The transport of ammonium/ammonia is a key process for the acquisition and metabolism of nitrogen. Ammonium transport is mediated by the AMT/MEP/Rh family of membrane proteins which are found in microorganisms, plants, and animals, including the Rhesus blood group antigens in humans. Although ammonium transporters from all kingdoms have been functionally expressed and partially characterized, the transport mechanism, as well as the identity of the true substrate (NH$_4^+$ or NH$_3$) remains unclear. Here we describe the functional expression and characterization of LeAMT1;1, a root hair ammonium transporter from tomato (Lycopersicon esculentum) in Xenopus oocytes. Micromolar concentrations of external ammonium were found to induce concentration- and voltage-dependent inward currents in oocytes injected with LeAMT1;1 cRNA, but not in water-injected control oocytes. The NH$_4^+$-induced currents were more than 3-fold larger than methylammonium currents and were not subject to inhibition by Na$^+$ or K$. The voltage dependence of the affinity of LeAMT1;1 toward its substrate strongly suggests that charged NH$_4^+$, rather than NH$_3$, is the true transport substrate. Furthermore, ammonium transport was independent of the external proton concentration between pH 5.5 and pH 8.5. LeAMT1;1 is concluded to mediate potential-driven NH$_4^+$ uptake and retrieval depending on root membrane potential and NH$_4^+$ concentration gradient.

Plasma membrane ammonium transport is critical for the acquisition and metabolism of nitrogen in many organisms (1). In aqueous solution, ammonium (NH$_4^+$) is in equilibrium with ammonia (NH$_3$) with a pK$_a$ of 9.25. At typical cytosolic pH, $\sim$99% is present in the cationic form. Uncharged NH$_3$ can pass lipid bilayers along its concentration gradient, but NH$_4^+$ is less permeable. As a consequence, both trans-membrane electrical and pH gradients affect NH$_3$/NH$_4^+$ equilibrium. In the following the term ammonium will be used to designate both the charged and uncharged species, and the chemical symbols will discriminate between NH$_4^+$ and NH$_3$.

Because of their acidic external environments, plant and yeast cells are anticipated to lose significant NH$_3$ derived from uptake and deamination processes. In contrast, the negative membrane potential of most cells favors NH$_4^+$ entry into cells along its electrochemical gradient. Thus NH$_4^+$ transporters generally represent uptake and scavenging systems for ammonium. Plants, yeast, and bacteria acquire ammonium by transporter proteins having high affinity for ammonium, whereas animals primarily use ammonium transporters for excretion.

Genetic studies in yeast revealed that protein-mediated ammonium transport is crucial for growth on low ammonium concentrations and identified a mutant deficient in ammonium uptake (2). Functional suppression of the growth defect of yeast strains deficient in ammonium uptake have led to the isolation of plant and yeast genes encoding high affinity ammonium transporters (3, 4). Six homologous genes (AMTs, Ammonium Transporters$^1$) were found in the genome of the plant Arabidopsis thaliana, three in tomato (Lycopersicon esculentum), and three in the yeast Saccharomyces cerevisiae (MEPs, Methylammonium Permeases) (5–9). Radiotracer uptake studies of the Arabidopsis ammonium transporters heterologously expressed in yeast have shown that the studied transporters possess affinities for ammonium in the nanomolar-micromolar range (3, 6). AMT/MEP proteins lack similarity to other well studied transporters and are highly hydrophobic membrane proteins with a predicted molecular mass of around 55 kDa and 11–12 putative transmembrane spans. Functionally, AMT proteins from Arabidopsis (AtAMT1;1, AtAMT1;2 and AtAMT1;3) behaved similarly to yeast MEP proteins regarding concentrative transport of both ammonium and methylammonium, dependence on metabolic energy and relative insensitivity to K$^+$ (3, 4, 6, 9). Related proteins from bacteria were also functionally characterized as ammonium transporters, e.g. from Corynebacterium glutamicum (10).

The mechanism of transport mediated by these transporters remains less clear. Several possibilities exist, including NH$_3$ diffusion, NH$_4^+$ uniport, and H$^+$-coupled symport mechanisms. Although electrical measurements are necessary to resolve the identity of the substrate (NH$_4^+$ or NH$_3$) transported by AMT-ammonium transporters, all studies relying on direct short term uptake measurements are consistent with net NH$_4^+$ transport (3, 4, 6, 9, 10). However, whether the transport is coupled to H$^+$ cotransport, although critical to understand ammonium fluxes and equilibrium, has not yet been resolved.

The growth defect of Escherichia coli and yeast mutants deficient in ammonium transport in media supplemented with low concentrations of ammonium as sole nitrogen source can be restored at higher pH (11, 12). Because at higher pH the relative proportion of NH$_3$ increases, the results led to the

$^1$ The abbreviations used are: AMT, ammonium transporter; HATS, high affinity transporter system; MEP, methylammonium permease; MES, 2-(N-morpholino)ethanesulfonic acid; Rh, rhesus.

$^*$ This work was supported by grants from the Bundesministerium für Bildung und Forschung and by the Deutsche Forschungsgemeinschaft Gottfried-Wilhelm Leibniz award (to W. B. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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suggestion that uncharged NH₃, but not NH₄⁺, is transported by bacterial AmtB and yeast MEP proteins (11, 12). In addition, the requirement for a proton gradient to sequester methyllumonioin in acidic vacuoles supported the interpretation that Amt/MEP/Rh proteins generally transport uncharged substrates (12).

Homologs of Amt/MEP have also been identified in animals, including several in humans. Two human homologs are mainly expressed in kidney, whereas others are specifically expressed in red blood cells (13–15). Interestingly, the erythrocyte homologs constitute the rhesus (Rh) polypeptides, a family of proteins that are core structural components of the Rh antigens. Despite their clinical importance in transfusion medicine, a physiological function as ammonium transporters has only recently been ascribed to Rh polypeptides (13). Rh protein complexes had earlier been known to be important for membrane integrity of red blood cells because individuals with the rare Rhnull disease (lack of Rh protein expression) display severe physiological function as ammonium transporters when expressed in yeast (13). Transport function of Rh proteins may be interpreted both as net ammonium influx and efflux, depending on the experimental conditions (13, 16).

In plants, ammonium uptake from the soil is mediated by high and low affinity systems (5, 17, 18). Plants generally prefer growth on soils containing both nitrate and ammonium, but because of the lower energetic cost needed for assimilation, ammonium may often be the preferred nitrogen source, as has been shown for e.g. Arabidopsis (6). The high affinity transporter systems (HATS) for ammonium are saturable in the micromolar range and are electrogenic (19–23). Additionally, low affinity ammonium transport systems (LATs) are not saturated even at millimolar concentrations (17, 18). The functional characteristics of HATS are similar to transporters of the Amt/MEP/Rh family. Thus, AMTs probably constitute the molecular basis of HATS (see below). In a thorough study in rice, uptake rates, membrane potentials, and cytosolic ammonium concentrations (in the mM range, determined by loading and efflux of [³²⁵]NH₄⁰) were estimated and led Wang et al. (23, 24) to suggest that, at least at low external ammonium concentrations, high affinity systems have to be secondarily active, e.g. ATP-dependent NH₄⁺ pumps or NH₃/H⁺ cotransporters. Exact cytosolic ammonium concentrations, as estimated by different techniques, however, have yielded controversial results (25). Relatively high concentrations (several mM) of ammonium have been estimated and measured in e.g. rice (26) and Chbara (27). However, because ammonium can collapse the pH gradient of tonoplast vesicles (28), high levels of ammonium in the cytosol may be cytotoxic, and consequently cytoplasmic ammonium levels are unlikely to be high. The transport mechanism by which plants acquire ammonium sets thermodynamic limits to cytosolic steady-state ammonium levels.

To investigate the biophysical properties of ammonium transporters of the Amt/MEP/Rh family, the plant ammonium transporter LeAMT1;1 was studied in more detail. The up-regulation of LeAMT1;1 transcripts in tomato root hairs under nitrogen deficiency suggests a major role for plant nutrition. We functionally expressed LeAMT1;1 heterologously in Xenopus oocytes and used a two-electrode voltage clamp to study the transport mechanism. Our findings indicate that NH₄⁺ uniport is the mechanism for LeAMT1;1.

**Ammonium Uniport in LeAMT1;1**

**Plasmid Construction**—LeAMT1;1 was amplified by PCR, restricted with appropriate enzymes, and ligated into pPO2, a pBF-derived oocyte expression plasmid (29) containing 5'- and 3'-untranslated β-globin sequences from Xenopus. pPO2 was derived from pBF by replacing the 40-bp poly(A) tail with a 92-bp poly(A) tail. Sequence was verified by sequencing. Capped cRNA was transcribed by SP6 RNA polymerase in vitro using Message Machine (Ambion Inc., Austin, TX), after linearization of the plasmid with MluI.

**Preparation and Injection of Oocytes—**Xenopus oocytes were removed from adult female frogs by surgery, manually dissected, and defolliculated. Oocytes (Dumont stage V or VI) were injected with 20–50 nl of cRNA (~20–50 ng/oocyte). Oocytes were kept at 16 °C in ND96 (in mM): 96 NaCl, 2 CaCl₂, 1.8 CaCl₂, 1 MgCl₂, 5 Hepes (pH 7.4), and gentamicyn (20 μg/ml).

**Electrophysiological Measurements—**Standard bath solutions contained (in mM): 100 NaCl, 2 CaCl₂, 2 MgCl₂, 4 Tris (pH adjusted to 7.5 with MES). For measurements at higher pH, the standard solution adjusted to pH 8.5 was used. For measurements at pH 5.5 and 6.5, the solution contained 4 mM MES, and the pH was adjusted using Tris.

To exclude effects of buffer substances on ammonium currents, solutions were used in which MES and Tris were replaced either with Hepes or phosphate. Induced currents were indistinguishable in solutions buffered with Tris, MES, Hepes, or phosphate buffer (data not shown). A few experiments were performed in low ionic strength solutions in the absence of monovalent cations. This solution “S” contained (in mM): 200 sucrose, 2 CaCl₂, 2 MgCl₂, 4 Hepes (pH 7.0), adjusted with Ca(OH)₂. In experiments with that solution the reference electrode was connected to the bath via an agar bridge. All experiments were repeated multiple times, and data were collected from more than nine batches of oocytes from different frogs.

**Ammonium potential and current measurements** were performed 2–5 days after injection using a Dagan CA1 amplifier and Axon pClamp6.0 software. For recordings as in Figs. 1 and 2, oocytes were voltage-clamped at constant negative potential, and currents were recorded continuously by a chart recorder. For all other measurements, oocytes were clamped to a holding potential (typically ~30 mV), and membrane currents were measured after stepping from holding to different test potentials. Short pulses (<200 ms) were used to keep slowly activating endogenous currents small.

**Conditions That Allow Measurement of Heterologously Expressed Transporters for Ammonium—**Ammonium is known to induce endogenous currents in uninjected oocytes (30, 31). Therefore, conditions were established which allow measurement of heterologously expressed transporters for ammonium. In agreement with published data (30, 31), at high ammonium concentrations (~100 mM) linear, time-independent currents were observed (data not shown). In contrast, at concentrations below 1 mM, ammonium did not affect or induce endogenous currents (see Figs. 1B and 2B). The absence of endogenous currents induced by low (~1 mM) ammonium concentrations was tested in all batches of oocytes prior to use.

Many batches of oocytes additionally showed a prominent, ammonium-independent endogenous inward current of more than 1-μA amplitude slowly activating at hyperpolarizing voltages, especially at low pH. At positive voltages, a variable and slowly activating outward oocyte current was often observed. Endogenous currents (especially in choline, see Fig. 6) limited the useful voltage range, so that generally pulses between +20 mV and ~140 mV in 20-mV steps were used. For each oocyte and each ammonium concentration, currents were measured alternatingly with and without substrate, and subtractive (induced) steady-state currents were determined by subtracting total currents.

**Data Analysis—**Data were processed using Sigmaplot (Jandel Scientific). Because of slightly variable expression levels in different batches of oocytes, representative induced currents from single oocytes are shown. When appropriate, the means ± S.E. values are displayed. Calculated affinities are given as the means ± S.D. The concentration dependence of ammonium-induced current at each voltage was fitted using Equation 1,

\[ I = I_{\text{max}}(1 + K_c/c) \]

(1)

where \( I_{\text{max}} \) is the maximal current at saturating ammonium concentration, \( K_c \) is the substrate concentration permitting half-maximal currents, and \( c \) is the experimentally used concentration. The voltage dependence of \( K_c \) was fitted with Equation 2,

\[ K_c(\delta) = K_c \exp(\delta \times e^2/2k_B T) \]

(2)

where \( \delta \) is the fractional electrical distance, \( e \) is the elementary charge, \( V \) is the membrane potential, \( k \) is Boltzmann’s constant, and \( T \) is the absolute temperature.
Ammonium Uniport in LeAMT1;1

RESULTS

LeAMT1;1 Transport Is Electrogenic—To determine whether uptake of ammonium is electrogenic, a two-electrode voltage clamp was used in *Xenopus* oocytes expressing the plant ammonium transporter LeAMT1;1. To avoid potential interference of ammonium transport and monovalent cations in the medium, measurements were performed initially in low ionic strength solutions in the absence of monovalent cations. Oocytes injected with cRNA encoding LeAMT1;1 were voltage-clamped at negative membrane potential and superfused with 100 μM ammonium. Low concentrations of ammonium reliably induced inwardly directed currents (Fig. 1A). The ammonium-induced current reversed to background levels after withdrawal of ammonium from the external solution. Repeated superfusion elicited nearly identical currents (Fig. 1A), whereas choline, sodium, or potassium induced only minute inward currents (Fig. 1A). The same solutions did not induce currents in control oocytes (Fig. 1B). Ammonium-induced currents (for magnitude and characteristics, see below) by LeAMT1;1 were virtually unchanged in solutions in which sucrose was replaced by sodium chloride. Because most studies in *Xenopus* oocytes use solutions containing sodium as the main cation, ammonium currents were nearly identical in sucrose and sodium solutions, and the use of sodium chloride solutions allowed stable recordings for several hours, all subsequent experiments were performed in solutions with 100 mM NaCl replacing 200 mM sucrose (Fig. 2, A and B). Occasionally we noted a nonstoichiometrically coupled linear background conductance associated with LeAMT1;1 expression and not seen in uninjected oocytes. The magnitude of this current was variable and linear and was not investigated in more detail.

Because high (mM) ammonium concentrations are known to induce endogenous currents in uninjected oocytes (30, 31), great care was taken to discriminate endogenous from LeAMT1;1-induced currents. All measurements were performed at concentrations <1 mM. At these concentrations ammonium did not affect endogenous background currents across the membrane of water-injected or noninjected oocytes (Figs. 1B and 2B). The absence of endogenous currents induced by low (<1 mM) ammonium concentrations was tested in all batches of oocytes prior to use (see “Experimental Procedures”). Specific ammonium-induced currents associated with LeAMT1;1 expression in voltage-clamped oocytes demonstrate electrogenic transport by LeAMT1;1. Inward flux of a charged substance should depolarize the membrane when not voltage-clamped. As expected and in accordance with LeAMT1;1-mediated ammonium currents, the membrane potential depolarized when LeAMT1;1-injected oocytes were superfused with ammonium (Fig. 2C), whereas ammonium did not mediate depolarization in uninjected control oocytes (data not shown).

**NH4**⁺ binds to a single site within the membrane electric field—Ammonium-induced currents in LeAMT1;1-injected oocytes were analyzed over a broad voltage range. Steady-state background currents were subtracted from total currents after adding ammonium to determine induced currents. Ammonium-induced currents by LeAMT1;1 were nearly instantaneous, with little time-dependent activation at potentials more negative than −100 mV, saturating at a steady state after several tens of milliseconds (Fig. 3). Currents were inwardly rectifying and increased with hyperpolarization and ammonium concentration (Figs. 3C and 4A). The shape of the current-voltage relation varied with ammonium concentration. At each voltage, currents showed simple saturation kinetics consistent with a Hill coefficient of 1, suggesting a single binding site (Fig. 4B). Interestingly, substrate concentrations permitting half-maximal currents, Kₘ, differed over the voltage range tested. The
FIG. 3. Voltage-dependent currents by LeAMT1;1. Shown are recordings of a LeAMT1;1-injected oocyte without ammonium (panel A) and in the presence of 100 μM ammonium (panel B). Panel C shows currents induced by 100 μM ammonium (panel B minus panel A). Induced currents were inwardly rectifying and reached a steady state after several tens of milliseconds. Currents at very negative potentials were activating slowly. The pulse protocol is shown in the inset; pulses ranged from 20 to −140 mV in 20-mV steps.

Panel A

Panel B

Panel C

FIG. 4. Ammonium currents in LeAMT1;1 are concentration- and voltage-dependent. Panel A, current-voltage relations of a representative single oocyte in different ammonium concentrations. Panel B, saturable ammonium-induced currents at different potentials. Shown are normalized means (to the same maximal current) ± S.D. from five different experiments. Values were fitted with Equation 1. Panel C, $K_m$ is voltage-dependent with the fractional electrical distance $\delta = 0.26 \pm 0.04 \ (n = 8)$.

$K_m$ was lower at more negative voltages, consistent with external NH$_4^+$ driven into a saturable binding site. Assuming a single binding site for NH$_4^+$, the voltage dependence of binding suggests that the site is situated ~26% (measured from external side) within the membrane electrical field (not translated into physical distance). The voltage dependence of the binding site supports that NH$_4^+$, not NH$_3$, is the substrate of LeAMT1;1 (see below).

LeAMT1;1 Is Selective for Ammonium and Methylammonium—To gain insight into the mechanism of selectivity, the transport capacity of LeAMT1;1 for structural analogs of ammonium was tested. Methylammonium (H$_3$C-NH$_3$) is used widely as a transport analog to measure ammonium transporter kinetics. When LeAMT1;1-injected oocytes were superfused with 100 μM methylammonium, about 5-fold smaller currents compared with ammonium were observed (Fig. 2). However, current-voltage characteristics were similar to ammonium-induced currents (Fig. 5A). Currents saturated (Fig. 5B), and the $K_m$ of LeAMT1;1 depended on membrane potential (Fig. 5C). Voltage dependence was similar ($\delta = 0.27$, compared with $\delta = 0.26$ for NH$_4^+$), suggesting a common saturable binding site for NH$_4^+$ and H$_3$C-NH$_3^+$, however with ~25-fold lower affinity to methylammonium (Fig. 5, B and C). Again, the results support that H$_3$C-NH$_3^+$, but not uncharged methylammonia (H$_3$C-NH$_2$, $K_a \approx 10.66$), is transported by LeAMT1;1. Methylammonia is structurally similar to the agronomically important cyanamide (N=C-NH$_2$). Consistent with the results above, superfusion of cyanamide at 100 μM at pH 7.5, where almost all cyanamide is in the uncharged form, did not induce currents or influence ammonium currents (Fig. 5D).

Potassium was also tested because it has an ionic radius and diffusion constant similar to those of NH$_4^+$ and because potassium channels are often capable of transporting both NH$_4^+$ and K$^+$ (32). At low concentrations (100 μM), K$^+$ induced a small current in LeAMT1;1-expressing oocytes (Fig. 2), but not in control oocytes. K$^+$ conductance was at least 25-fold lower than NH$_4^+$, suggesting that LeAMT1;1 is able to discriminate efficiently between NH$_4^+$ and potassium. Furthermore, NH$_4^+$-elicited currents were unaffected by a >1,000-fold excess of monovalent cations. Current-voltage curves, as well as the magnitude of induced currents and calculated affinities, were very similar in the presence of 100 mM sodium, potassium, and choline (Fig. 6), indicating that ammonium transport in LeAMT1;1 is largely independent of other alkali cations in the external medium.

Ammonium Transport Is Unaffected by External pH Changes—The results described so far do not allow differentiation between transport of NH$_4^+$ and H$^+$/NH$_4^+$ cotransport. To determine whether ammonium transport by LeAMT1;1 is mediated via passive diffusion or secondary active H$^+$/NH$_4^+$ cotransport, ammonium-induced currents were measured at different external proton concentrations, varying the pH from 5.5 to 8.5. For optimal buffering capacity, different buffer substances were used. Comparison of induced currents at pH 7.5 in the presence of a variety of different buffers did not show significant effects on the background or induced currents (see “Experimental Procedures”). Although proton availability changed 1,000-fold, the current magnitude induced by 500 μM ammonium was changed by less than 5% (Fig. 7A). In addition, the $K_m$ for NH$_4^+$...
was independent of external pH at all voltages tested (Fig. 7B). These findings argue strongly against NH₃ as the transport substrate because the remarkably unchanged current magnitudes in the pH range tested would require a compensating shift in the affinity of the transporter for NH₃ by 3 orders of magnitude.

At all voltages and concentrations tested, transport of NH₄⁺ was independent of pH. In addition, the reversal potential of total currents was insensitive to a 1,000-fold shift in proton concentration (Fig. 7C) but was sensitive only to changes in external ammonium concentration.

DISCUSSION

LeAMT1;1 Is an Ammonium Uniporter—To date, conclusions drawn on the nitrogen form transported by AMT/MEP/Rh-type transport proteins (i.e. NH₄⁺ versus NH₃) were based mostly on
pH-dependent growth and methylammonium uptake experiments in yeast and bacteria. In addition, electrophysiological studies on algae/plant cells expressing many ammonium transporters indicated the electrogenic nature of high affinity ammonium transport.

This study presents the successful heterologous expression and electrophysiological characterization of a single ammonium transporter in *Xenopus* oocytes and provides several lines of evidence that NH\textsubscript{4}+/H\textsuperscript{+} uniport is the mechanism of transport mediated by LeAMT1;1. First, ammonium and methylammonium induced concentration- and voltage-dependent currents, suggesting a single NH\textsubscript{4}+/H\textsuperscript{+} (and H\textsubscript{3}C-NH\textsubscript{3}/H\textsuperscript{+}) binding site within the membrane electric field of the transport protein. Repeated superfusion elicited nearly identical currents, suggesting that influx of ammonium did not substantially change internal pH or ammonium concentrations and that changes in these parameters did not influence transport activity. Second, NH\textsubscript{4}+-induced currents mediated by LeAMT1;1 were independent of pH. If the transport mechanism of LeAMT1;1 were a NH\textsubscript{4}+/H\textsuperscript{+} cotransport mechanism, reversal of NH\textsubscript{4}+/H\textsuperscript{+} currents would be predicted to change by more than 170 mV between pH 5.5 and 8.5 (assuming a 1 H\textsuperscript{+}:1 NH\textsubscript{4}+ stoichiometry). In addition, inward directed ammonium currents were detected at low external ammonium at every pH measured, even at pH 8.5. Assuming an oocyte bathed in pH 8.5 with an internal pH of 7.35 (31) and a H\textsuperscript{+}/NH\textsubscript{4}+ cotransport mechanism, the more than 10-fold higher internal H\textsuperscript{+} concentration would counteract the inward directed driving force for ammonium and thus should diminish inward currents. The results strongly support a proton-independent ammonium transport mechanism. In addition, LeAMT1;1 was highly selective for NH\textsubscript{4}+ but also permeated H\textsubscript{3}C-NH\textsubscript{3} and displayed very small conductances for other monovalent cations (Figs. 1 and 2). This is in agreement with uptake studies in yeast, where AMT/MEP-mediated methylammonium uptake was almost unaffected by a 5-fold molar excess of K\textsuperscript{+} and was reduced only 30% by a 200-fold molar excess of K\textsuperscript{+} (3, 4).

In the yeast *S. cerevisiae*, biochemical transport properties of AtAMTs (3, 6), LeAMTs,\textsuperscript{2} and MEPs (4, 9) were similar, suggesting a common transport mechanism for these proteins. LeAMT1;1 currents were nearly unchanged when NH\textsubscript{3} and methylammonia concentrations increased by orders of magnitude (pH 5.5 compared with pH 8.5). The cations are not distinguished merely by size exclusion and optimized de- and rehydration properties because potassium, which has a size similar to that of ammonium and behaves similarly in solution, is excluded efficiently by LeAMT1;1. Efficient transport of methylammonium may suggest that chemical interactions with NH\textsubscript{4}+ and the amino group of H\textsubscript{3}C-NH\textsubscript{3} play an important role.

\textsuperscript{2} U. Ludewig, N. von Wirén, and W. B. Frommer, unpublished data.
role in substrate recognition. Analysis of AtAMT1;1 in yeast revealed a maximum of methylammonium uptake at around pH 7 (3). However, these findings do not contradict the pH independence of LeAMT1;1 in oocytes because LeAMT1;1 is strongly voltage-dependent and because the lower uptake observed in yeast under similar imposed conditions may be explained by a less hyperpolarized plasma membrane at acidic external pH (33). The lower uptake rates in yeast at alkaline external pH may be also because of less hyperpolarized membrane or because of changed compartmentation of ammonium at alkaline external pH. The ammonium and methylammonium transport mechanism of the more distantly related Rh proteins may be different (13, 16), but Rh proteins were also found to mediate net ammonium uptake (13).

NH$_4^+$ uniport as the mechanism for ammonium transport has also been described for Chara and Nitella (20, 21). Interestingly, both aquatic plants showed a voltage dependence of currents and affinities for ammonium similar to those of LeAMT1;1. These results were interpreted as saturable binding of NH$_4^+$ within 30% of the membrane electrical field. In addition, it was concluded from combined electrophysiological and radioactive tracer measurements that the transport mechanism of these algal uptake systems is NH$_4^+$ uniport (20, 21). NH$_4^+$ uniport also best explained the transport properties determined for the related bacterial ammonium transporters, i.e. from C. glutamicum (10).

Growth studies with yeast and bacterial ammonium transporter deletion strains suggested that ammonium uptake in yeast and E. coli is dependent on these common transporters only at acidic pH (5.5) but not at pH 7.5 (11, 12). Using vascular proton pump yeast mutants Soupene et al. (12) observed that [$^{14}$C]methylammonium long term uptake was diminished relative to the wild type, whereas uptake of [$^{14}$C]-sugars was not. This was interpreted in favor of NH$_4^+$/NH$_3$-CH$_2$ plasma membrane transport, with subsequent vacuolar accumulation of methylammonium depending on vacuolar acidification. However, growth tests only very indirectly address the transport mechanism in ammonium transporters and are sensitive to secondary effects, e.g. changes in membrane potential or compartmentation. Moreover, the reduction of [$^{14}$C]methylammonium uptake in yeast strains defective in vascular acidification is difficult to interpret in terms of the nitrogen form taken up because cytosolic pH and membrane potential in mutant strains may be different compared with wild type. Altered methylammonium uptake by mutant yeast may also be explained by other secondary effects. If methylammonium is not accumulated in the vacuole, cytosolic methylammonium concentrations are increased, and thus passive methylammonium influx via plasma membrane MEP transporters is lowered because of the unfavorable methylammonium plasma membrane gradients.

Electrophysiological studies on whole root cells as well as single algal cells showed a strong depolarization in response to ammonium supply, very similar to the depolarization exhibited by LeAMT1;1 (19–23). In addition, ammonium uptake by HATS was relatively pH-independent between pH 4.5 and pH 9 (18), similar to our observations with LeAMT1;1 in oocytes (Fig. 7).

Taken together, NH$_4^+$ transport properties, as described in earlier electrophysiological studies on intact plant cells, are in agreement with the present characterization of LeAMT1;1-mediated NH$_4^+$ transport, both arguing in favor of NH$_4^+$ uniport.

**Physiological Significance of the NH$_4^+$ Uniport Mechanism**—Based on electrophysiological and short term uptake studies using labeled NH$_4^+$, high and low affinity systems for ammonium uptake in plants can be distinguished. The results presented here suggest differentiation of transport systems by their mechanism of transport. Low affinity systems described as voltage-independent have not yet been molecularly isolated and are clearly different from voltage-dependent transporters such as LeAMT1;1 in their transport mechanisms (17, 23). Plant HATS have been assumed to be active uptake systems, likely primary active NH$_4^+$ pumps or H+/NH$_4^+$ cotransporters because of their ability to concentrate millimolar cytosolic ammonium in low external ammonium concentrations (23). Because the sequences of LeAMT1;1 and other AMTs do not contain an obvious ATP binding/nucleotide binding site, a primary active pump mechanism seems very unlikely to apply for AMT/MEP/Rh ammonium transport. The perceived necessity of an active HATS for ammonium uptake, however, was largely the result of estimated high cytosolic ammonium concentrations (24). Exact cytosolic ammonium concentrations are still a matter of debate, although evidence is accumulating that cytosolic ammonium concentrations are in the mM range (25–27). If AMTs constitute a major component of HATS, NH$_4^+$ uniport would allow passive flux along its electrochemical concentration gradient. In this case, membrane potential imposes upper limits on cytosolic ammonium concentrations and may require reevaluation of estimated cytosolic ammonium concentrations.

Studies with metabolic inhibitors, both on intact plant roots and on yeast, indicated the importance of the proton gradient for ammonium uptake (3, 4, 23). However, metabolic inhibitors not only decrease ATP synthesis and proton gradient, but also lead to breakdown of membrane potential. Likewise, protonophores not only collapse the proton gradient, but also cause the membrane potential, which is largely the result of protons in yeast, to diminish. Because NH$_4^+$ transport by LeAMT1;1 is strongly voltage-dependent, metabolic inhibitors and protonophores are expected to decrease ammonium uptake in plants and yeast (3, 4, 23).

In uptake studies using roots from different tomato cultivars grown in 50 μM NH$_4^+$, both ammonium and methylammonium were shown to be taken up by a system with affinity for ammonium of ~8.5 μM (34, 35), a value obtained here for LeAMT1;1 at –140 mV (Fig. 4). The affinity for methylammonium uptake was at least 1 order of magnitude lower, consistent with the data presented here. Thus in cultivated tomato, under the conditions used by Smart and Bloom (34), LeAMT1;1 may represent the main pathway for ammonium uptake. Among the LeAMTs, both LeAMT1;1 and LeAMT1;2 are expressed in roots and especially in the plasma membrane of root hairs (7). Thus root hairs may participate in ammonium uptake from the soil (36, 37). Ammonium transporter expression is regulated at the transcriptional and posttranscriptional level by the nitrogen source (7, 8). LeAMT1;1 transcripts are induced by nitrogen deficiency, whereas LeAMT1;2 expression increases after NH$_4^+$ or NO$_3^-$ supply (8). Thus, at low ammonium concentrations, LeAMT1;1 may be expected to be mainly responsible for ammonium uptake, whereas LeAMT1;2 is expected to contribute at high ammonium concentrations.

Under most physiological conditions, electrochemical gradients will be inwardly directed, leading to NH$_4^+$ accumulation. In addition, NH$_4^+$ passively diffusing out of the cell may be retrieved upon reprotonation to NH$_4^+$ from the more acidic apoplast. At depolarized potentials or at conditions leading to high cytosolic NH$_4^+$ concentrations, however, passive NH$_4^+$ fluxes may be directed outward, serving as a way to protect cells from accumulation of cytotoxic ammonium levels.

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3 S. Wilken, W. B. Frommer, and N. von Wirén, manuscript in preparation.
Acknowledgments—We thank Wolfgang Jost and Mechthild Linnemann for excellent technical assistance, Stefan Gruß for the oocyte expression plasmid, and Gene Kim for critical reading the manuscript.

REFERENCES
1. Kleiner, D. (1981) Biochim. Biophys. Acta 639, 41–52
2. Dubois, E., and Grenson, M. (1979) Mol. Gen. Genet. 175, 67–76
3. Ninnemann, O., Jauniaux, J. C., and Frommer, W. B. (1994) EMBO J. 13, 3464–3471
4. Marini, A. M., Vissers, S., Urrestarazu, A., and André (1994) EMBO J. 13, 3456–3463
5. von Wirén, N., Gazzarrini, S., Gojon, A., and Frommer, W. B. (2000) Curr. Opin. Plant Biol. 3, 254–261
6. Gazzarrini, S., Lejay, L., Gojon, A., Ninnemann, O., Frommer, W. B., and von Wirén, N. (1999) Plant Cell 11, 937–948
7. Lauter, F. R., Ninnemann, O., Bucher, M., Riesmeier, J. W., and Frommer, W. B. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8139–8144
8. von Wirén, N., Lauter, F. R., Ninnemann, O., Gillissen, B., Walch-Liu, P., Engels, C., Jost, W., and Frommer, W. B. (2000) Plant J. 21, 167–175
9. Marini, A. M., Soussi-Boudekou, S., Vissers, S., and André, B. (1997) Mol. Cell. Biol. 17, 4282–4290
10. Meier-Wagner, J., Nolden, L., Jakoby, M., Siewe, R., Krämer, R., and Burkovski, A. (2001) Microbiology 147, 135–143
11. Soupene, E., He, L., Yan, D., and Kustu, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7030–7034
12. Soupene, E., Ramires, R. M., and Kustu, S. (2001) Mol. Cell. Biol. 21, 5733–5741
13. Marini, A. M., Matassi, G., Raynal, V., André, B., Cartron, J. P., and Cherif-Zahar, B. (2000) Nat. Genet. 26, 341–344
14. Liu, Z., Peng, J., Mo, R., Hui, C. C., and Huang, C. H. (2001) J. Biol. Chem. 276, 1424–1433
15. Avent, N. D., and Reid, M. E. (2000) Blood 95, 375–387
16. Ludewig, U., von Wirén, N., Rentsch, D., and Frommer, W. B. (2001) Genome Biol. 2, reviews 1010.1–1010.5
17. Ullrich, W. R., Larsson, M., Larsson, C. M., Lesch, S., and Novacky, A. (1984) Physiol. Plant. 61, 369–376
18. Wang, M. Y., Siddiqi, M. Y., Ruth, T. J., and Glass, A. D. M. (1993) Plant Physiol. 103, 1259–1267
19. Higinbotham, N., Etherton, B., and Foster, R. J. (1964) Plant Physiol. 39, 196–203
20. Walker, N. A., Beilby, M. J., and Smith, F. A. (1979) J. Membr. Biol. 49, 21–55
21. Walker, N. A., Smith, F. A., and Beilby, M. J. (1979) J. Membr. Biol. 49, 238–296
22. Felle, H. (1968) Biochim. Biophys. Acta 602, 181–185
23. Wang, M. Y., Glass, A. D. M., Shaff, J. E., and Kochian, L. V. (1994) Plant Physiol. 104, 899–906
24. Wang, M. Y., Siddiqi, M. Y., Ruth, T. J., and Glass, A. D. M. (1993) Plant Physiol. 103, 1249–1258
25. Britto, D. T., Glass, A. D., Kronzucker, H. J., and Siddiqi, M. Y. (2001) Plant Physiol. 125, 523–526
26. Britto, D. T., Siddiqi, M. Y., Glass, A. D., and Kronzucker, H. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4255–4258
27. Wells, D. M., and Miller, A. J. (2000) Plant Soil 221, 105–108
28. Garbarino, J., and Dupont, F. M. (1998) Plant Physiol. 116, 231–236
29. Baurkowitz, T., Tucker, S. J., Schulte, U., Benndorf, K., Ruppersberg, J. P., and Falkner, B. (1999) EMBO J. 18, 847–853
30. Burchhardt, B. C., and Burchhardt, G. (1997) Pflügers Arch. Eur. J. Physiol. 434, 306–312
31. Cougnon, M., Bouyer, P., Hulin, P., Anagnostopoulos, T., and Planelles, G. (1996) Pflügers Arch. Eur. J. Physiol. 431, 658–667
32. Moroni, A., Bardella, L., and Thiel, G. (1998) J. Membr. Biol. 163, 25–35
33. Madrid, R., Gomez, M. J., Ramos, J., and Rodriguez-Navarro, A. (1998) J. Biol. Chem. 273, 14388–14444
34. Smart, D. R., and Bloom, A. J. (1988) Oecologia 76, 336–340
35. Kosola, K. R., and Bloom, A. J. (1994) Plant Physiol. 105, 435–442
36. Henriksen, G. H., Raman, D. R., Walker, L. P., and Spanswick, R. M. (1992) Plant Physiol. 99, 734–747
37. Colmer, T. D., and Bloom, A. J. (1998) Plant Cell Environ. 21, 240–246
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J. Biol. Chem. 2002, 277:13548-13555.
doi: 10.1074/jbc.M200739200 originally published online January 30, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M200739200

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