K^+ channel profile and electrical properties of *Arabidopsis* root hairs

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Abstract **Ion channels and solute transporters in the plasma membrane of root hairs are proposed to control nutrient uptake, osmoregulation and polar growth. Here we analyzed the molecular components of potassium transport in *Arabidopsis* root hairs by combining K^+-selective electrodes, reverse transcription-PCR, and patch-clamp measurements. The two inward rectifiers AKT1 and ATKC1 as well as the outward rectifier GORK dominated the root hair K^+ channel pool. Root hairs of AKT1 and ATKC1 loss-of-function plants completely lack the K^+ uptake channel or exhibited altered properties, respectively. Upon oligochitin-elicitor treatment of root hairs, transient changes in K^+ fluxes and membrane polarization were recorded in wild-type plants, while *akt1-1* root hairs showed a reduced amplitude and pronounced delay in the potassium re-uptake process. This indicates that AKT1 and ATKC1 represent essential α-subunits of the inward rectifier. Green fluorescent protein (GFP) fluorescence following ballistic bombardment with GORK promoter-GFP constructs as well as analysis of promoter-GUS lines identified this K^+ outward rectifier as a novel ion channel expressed in root hairs. Based on the expression profile and the electrical properties of the root hair plasma membrane we conclude that AKT1-, ATKC- and GORK-mediated potassium transport is essential for osmoregulation and repolarization of the membrane potential in response to elicitors.** **© 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Root hairs; Potassium channel; Nod factor; Channel-gating; Patch-clamp; Ion-sensitive microelectrodes

1. Introduction

Apart from being antennas of the rhizosphere with whom the plants experience encounters with micro-organisms, root hairs are a primary site for uptake of nutrients and water. To gain insight into the nature of the ion transport processes of single root hairs, patch-clamp, discontinuous single-electrode voltage-clamp techniques, and ion-selective electrodes were applied ([1–3] and references therein). Upon hyperpolarization slow-activating, inward rectifying channels were identified as the major pathways for the influx of potassium ions in young growing root hairs [2]. In search for transporters involved in potassium uptake of tomato roots, Hartje et al. [4] identified LKT1, an AKT1 orthologue of *Arabidopsis*. In line with the dominant role of this inward K^+ rectifier, *Arabidopsis* mutants impaired in AKT1 function exhibited a pronounced growth retardation [5]. In addition to these members of the AKT1 family, recently Downey et al. [6] localized KDC1 in carrot root hairs, which together with ATKC1 from *Arabidopsis* forms a separate branch within the plant *Shaker* K^+ channel family [7].

Besides their role in nutrient uptake epidermal cells are involved in the perception and transduction of environmental signals [8]. Among them root hair cells e.g. respond to salt and osmotic stress [9], mechanical stimuli [10], microbial factors [11] as well as phytohormones [12]. The earliest detectable response of root hair cells to external signals is a Ca^{2+} influx, the activation of anion channels, and in turn a depolarization of the plasma membrane [1,13]. Potassium efflux, which accompanies this depolarization, is thought to occur via depolarization-activated K^+ channels, which have been recorded in different root tissues ([14] and references therein).

Recently, the *Arabidopsis thaliana* guard cell outward rectifying K^+ channel (GORK) was identified, cloned and functionally characterized in *Xenopus* oocytes [15]. Together with SKOR (stelar K^+ outward rectifier, [16]), PTORK (*Populus tremula* outward rectifying K^+ channel) and SPORK (*Samanea saman pulvinus* outward rectifying K^+ channel), GORK forms a subfamily of *Shaker*-like plant potassium channels.

Given the fact that the *Arabidopsis* genome encodes nine *Shaker*-like K^+ channels, we in this study focused on the molecular and biophysical characterization of the plasma membrane K^+ channels in root hairs of this model plant. Thereby we demonstrate that root hairs of *A. thaliana* express AKT1, ATKC1, and GORK, and provide an in vivo electrophysiological characterization of the outward and inward rectifying K^+ channels identified in wild-type plants and K^+ channel loss-of-function mutants.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of *A. thaliana* ecotype Columbia were surface-sterilized in 70% ethanol and 5% NaClO+0.01% Triton X-100, and germinated on a filter paper moistured with Murashige and Skoog medium, supplemented with 2% sucrose. Seedlings were grown under sterile conditions in a growing chamber at 8/16 h day/night regime, 21/16°C day/night temperature, 80% relative humidity and photon flux density 100 μmol m^{-2} s^{-1}. $(GlcNAc)_8$ was purchased from Sigma.

2.2. Protoplasts isolation

Protoplasts were isolated from 7–8-day-old primary roots. The en-

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zyme solution contained 0.8% (w/v) cellulase (Onozuka R-10), 0.1% pectolyase (Sigma), 0.5% bovine serum albumin, 0.5% polyvinylpyrrolidone, 1 mM $CaCl_2$, 8 mM Mes/Tris, pH 5.6. Osmolarity of the enzyme solution was adjusted to 280 mosmol kg^{-1} using D-sorbitol. Roots were incubated in enzyme solution at 30°C during 30 min. Protoplasts released from the tip of the root hairs were filtered through 50 μm nylon mesh and washed in 1 mM $CaCl_2$ buffer (osmolarity 280 mosmol kg^{-1}, pH 5.6) by centrifugation twice (10 min at 500 rpm and 4°C). The protoplast suspension was stored on ice and aliquots used for reverse transcription (RT)-PCR and patch-clamp measurement.

2.3. RT-PCR experiments

For RT-PCR analyses, root hair protoplasts were isolated as described above and mRNA was purified twice with the Dynabeads mRNA Direct kit (Dynal, Oslo, Norway) to minimize DNA contaminations. First strand cDNA synthesis and RT-PCR were performed as described previously [21].

2.4. Transient expression assay

The *GORK* promoter was cloned into the pCRII-TOPO-TA vector (Invitrogen) as a 1525 bp PCR fragment, using the primer pair GORK-Ps (5′-ATTGTTATAGATCACTTTAAGAGTGATTACATTC-3′), GORK-Pas (5′-GTTTTCAAGAATCGGTTAAATGAAATC-3′). The smRS-green fluorescent protein (GFP) [32] was cloned as a *Bam*HI/*Eco*RI fragment into pUC18 to result into pUC-RSGFP. Following sequence verification the GORK promoter from pCRII-TOPO was cloned as an *Xho*I/*Bam*HI fragment into pUC-RSGFP via *Sal*I/*Bam*HI, creating pUC-GORKP-GFP, which was used in transient expression studies.

A self-made helium-driven ballistic particle delivery system (PIG: Particle Inflow Gun) was employed for transient expression studies. The concentration of the pUC-GORKP-GFP plasmid DNA was adjusted to 1 μg ml^{-1} and precipitated on gold particles (0.6 μm diameter on average, Bio-Rad) serving as microcarriers. In brief, gold particles (50 mg) were washed in 0.5 ml ethanol, dried and resuspended in water to a final concentration of 100 μg ml^{-1}. To 50 μl of gold particle solution an equal volume of sterile water was added. Subsequently the following components were added under continuous shaking: 20 μl plasmid DNA, 100 μl 2.5 M $CaCl_2$ and 40 μl 0.1 M spermidine. After vigorous shaking for 3 min, 200 μl cold ethanol was added and following mixing another 400 μl ethanol was added. DNA was precipitated on ice for 30 min and centrifuged briefly, the supernatant was removed and the DNA-covered gold particles were resuspended in 100 μl sterile H_2O. For particle bombardment, 10 μl of this solution per trial was used (i.e. 2 μg DNA and 500 μg gold particles). Forty-eight hours after bombardment (-6×10^4 Pa vacuum and 9.5×10^5 Pa pressure) seedlings were inspected for GFP expression on a confocal laser-scanning microscope.

2.5. Heterologous expression in Xenopus oocytes

GORK cRNA was prepared using the mMESSAGE mMACHINE™ RNA Transcription kit (Ambion). Preparation of oocytes and injection of cRNA have been described before [15]. Whole-cell currents were recorded using the two-electrode voltage-clamp technique. Oocytes were perfused with solution containing 30 mM K-gluconate, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM Tris/Mes pH 5.6. Osmolarity was adjusted to 220 mosmol kg^{-1} using D-sorbitol.

2.6. Patch-clamp recordings

Measurements were performed in whole-cell and outside-out mode using an EPC-7 patch-clamp amplifier (List-Medical-Electronic, Darmstadt, Germany). Data were low-pass-filtered with an eight-pole Bessel filter with a cut-off frequency of 2 kHz and sampled at 2.5 times the filter frequency. Data were digitized (ITC-16, Instrutech Corp., Elmont, NY, USA), stored on hard disk and analyzed using software PULSE and PULSEFIT (HEKA Elektronik, 1999), and IGORPro (WaveMetrics Inc., 1999). Patch pipettes were prepared from Kimax-51 glass capillaries (Kimble products, Vineland, NY, USA) and coated with silicone (Sylgard 184 silicone elastomer kit, Dow Corning, USA). The command voltages were corrected off-line for liquid junction potentials [33]. Pipette solutions (cytoplasmic side) contained: 150 mM K-gluconate, 2 mM $MgCl_2$, 10 mM EGTA, 2 mM Mg-ATP, 10 mM HEPES/Tris (pH 7.4). The standard external solutions contained: 30 mM K-gluconate, 1 mM $CaCl_2$, 10 mM Mes/Tris (pH 5.6) or HEPES/Tris (pH 7.4). Osmolarity of the solutions was adjusted to 280 mosmol kg^{-1} using D-sorbitol. Modifications in solute compositions are included in the figure legends. Chemicals were obtained from Sigma. The *skor1-1* (Stock No. N3816) and the *akt1-1* (Stock No. N3762) mutant lines were obtained from NASC (http://nasc.nott.ac.uk/).

2.7. Biophysical analysis

The voltage-dependence of the outward rectifier was described by its relative open probability (p_o). In a double-voltage step protocol, time- and voltage-dependent outward currents (I) were induced during a first activation pulse to depolarizing voltages (V). During a second pulse to negative voltage, the current changed direction to inward and then slowly deactivated. The I_0–V relationship obtained from extrapolation the deactivation time course of the second pulse to $t=0$ ms with an exponential function is proportional to the relative open probability of the channel at the end of activation pulse ($I_0 = N\cdot i\cdot p_o$), where N denotes the number of channels, I_0 the instantaneous tail current, and i the single-channel current at the fixed voltage of the second pulse. The relation p_o to V was fitted by a Boltzmann function:

$$p_o = 1/(1+e^{(V-V_{1/2}/V_S)}) \quad (1)$$

Here V_S denotes the slope factor, which correlates to the apparent gating charge and $V_{1/2}$ the half-activation voltage.

The sigmoidal time course of activation was fitted to the Hodgkin and Huxley model. According to the model, current activation was described by the relation [34]:

$$I(t) = I_{leak} + I(1-\exp(-t/\tau_{act}))^p \quad (2)$$

where I_{leak} is the leak current, I is a maximum activation current carried by the channel at a given voltage, τ_{act} is the time constant for activation and p is the number of independent gating particles.

2.8. K^+-selective microelectrodes and membrane potential measurements

The set-up for membrane potential measurements on root hairs and the fabrication of ion-sensitive microelectrodes have been described in detail previously [1,17].

3. Results

3.1. Elicitors trigger root hair K^+ release and re-uptake

Root hairs increase the root surface area in contact with the soil and represent a major site for nutrient uptake and plant–microbe interaction [8]. In the latter process plant and microbe-derived factors, so-called elicitors, trigger plasma membrane transporters, early events which can be detected with electrophysiological means.

To study potassium fluxes across the plasma membrane of root hairs K^+-selective electrodes were brought close to root hairs from 5–10-day-old *Arabidopsis* seedlings. In the presence of 10–20 μM potassium in the perfusion medium steady-state K^+ activity around 20–30 μM was recorded (Fig. 1, WT), indicating that K^+ uptake and release balanced each other. Following the application of 1 μM $(GlcNAc)_8$, K^+ efflux increased, reached a peak 12–13 min after stimulus onset, before K^+ re-uptake provided for a slow recovery of the apoplastic K^+ concentration. In parallel experiments root hairs were impaled with microelectrodes and the response of the root hair plasma membrane potential to $(GlcNAc)_8$ was monitored. In the presence of 10 μM potassium in the perfusing solution resting potentials as negative as −200 to −240 mV were recorded (Fig. 1, E_m (WT)). Upon elicitor treatment, the membrane depolarized by 30–60 mV before it slowly repolarized again. This transient elicitor-induced membrane depolarization showed a similar time course as the K^+ concentration changes. Given a cytosolic root hair K^+ concentration of about 80–100 mM (as determined by measuring the diffusion

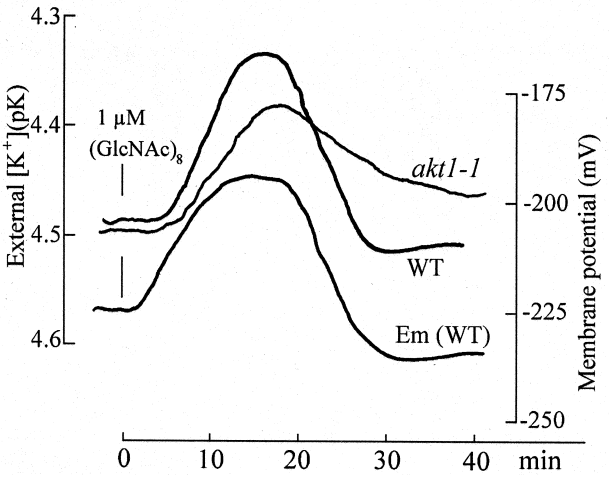

Fig. 1. $(GlcNAc)_8$ elicits membrane depolarization and K^+ efflux in root hairs. K^+ activity (pK_o) and membrane potential (E_m) measured in the root hair space of *A. thaliana* wild-type (WT) and mutant (*akt1-1*) before and after external addition of 1 μM $(GlcNAc)_8$ to the perfusion medium. Seedlings were fixed with candle wax on the bottom of a flow-through chamber and adapted to the solution for approximately 2 h. To detect K^+ activity, a K^+-selective microelectrode was placed between root hairs and K^+ activity was recorded before adding the test solution to allow for adaptation to the basic solution. E_m and K^+ measurements were carried out simultaneously on the same root. Kinetics shown are those that are closest to the statistical means. WT: maximal $\Delta[K^+] = 10.5 \pm 3.9$ μM (S.E.M.; $n = 10$); AKT1: maximal $\Delta[K^+] = 8.5 \pm 1.9$ μM (S.E.M.; $n = 8$).

potential at different external K^+ concentrations) and measuring 20–30 μM K^+ in the cell wall (Fig. 1) a Nernst potential for potassium (E_{K^+}) of −202 to −218 mV can be calculated. Thus the elicitor depolarizations – positive to E_K – indicate that in the initial phase Ca^{2+} and Cl^- pass the plasma membrane [17]. In this context it should be mentioned that elicitors and Nod factors have been shown to cause Ca^{2+} influx [18], respectively the activation of calcium-permeable channels [19]. Since the Nernst potentials for calcium and chloride are positive, the termination of the depolarization towards E_K seems thus to result from K^+ release. Accordingly, the efflux of positive charge balances the efflux of anions and initiates repolarization. Later H^+ pump-driven hyperpolarization and K^+ uptake seem to restore the resting potential.

3.2. AKT1 and ATKC1 co-express in root hairs

To investigate the molecular basis of channel-mediated K^+ uptake and release of root hairs we analyzed mRNA derived from *Arabidopsis* root hair protoplasts (Fig. 2A,B) by RT-PCR techniques (Fig. 2C). When using specific oligonucleotides directed towards the nine *Shaker*-like K^+ channels encoded by the *Arabidopsis* genome, we could demonstrate the expression of three K^+ channel transcripts in this particular cell type (Fig. 2C). In line with their role as an important site for nutrient supply and related studies on tomato and carrot, *Arabidopsis* root hairs expressed the inward rectifying K^+ channels AKT1 (Fig. 2C, cf. [4,20]) and ATKC1 (Fig. 2C, cf. [6]).

To prove the predicted role of AKT1 for K^+ re-uptake in the last phase of the root hairs elicitor response, we analyzed $(GlcNAc)_8$-treated root hairs of the AKT1 loss-of-function mutant *akt1-1*. Following elicitor application, both potassium release and re-uptake could be monitored by K^+-selective electrodes. In the mutants root hairs, however, peak K^+ release and the re-uptake rates were largely reduced (Fig. 1, *akt1-1*). This indicates that AKT1 is not only required for K^+-dependent root growth but also represents a membrane-delimited component of the elicitor response.

3.3. GORK represents a new root K^+ channel expressed in root hairs

As a third root hair K^+ channel transcript, we identified the outward rectifier GORK (Fig. 2C, cf. [15]). To confirm epidermal cells as sites of GORK expression, we fused the GORK promoter to GFP and performed transient expression

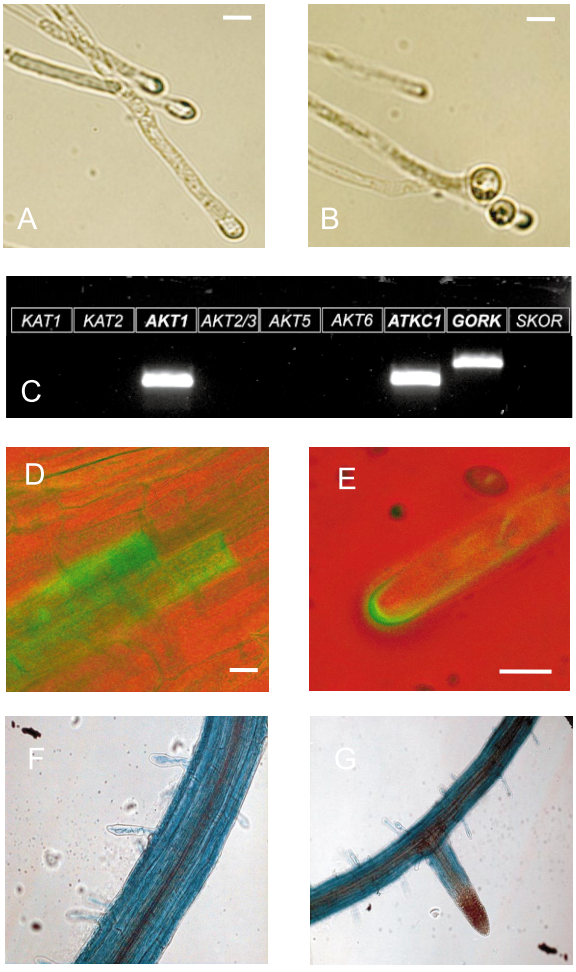

Fig. 2. Isolation of root hair protoplasts and K^+ channel expression. Release of protoplasts from root hairs of *A. thaliana* during enzymatic digestion after 5 (A) and 30 min (B) of treatment. C: Gel electrophoresis of RT-PCR products from *A. thaliana* root hair protoplast mRNA; representative of $n = 3$ experiments. D–G: The *GORK* promoter is active in root epidermal cells. A confocal laser-scanning microscope was used to follow transient GFP expression driven by the *GORK* promoter following ballistic particle bombardment of 7-day-old *A. thaliana* seedlings. GFP expression is visible after 48 h in epidermal root cells and is maintained during differentiation and outgrowth of root hairs (E). Photographs represent merged images of consecutive scans in transmission and epifluorescence mode; background in false color, red. Bars = 10 μm. GUS-staining of roots and root hairs of *Arabidopsis* seedlings expressing the *uidA* gene under control of the GORK promoter (F, G). Note the absence of GUS activity in root tips in G.

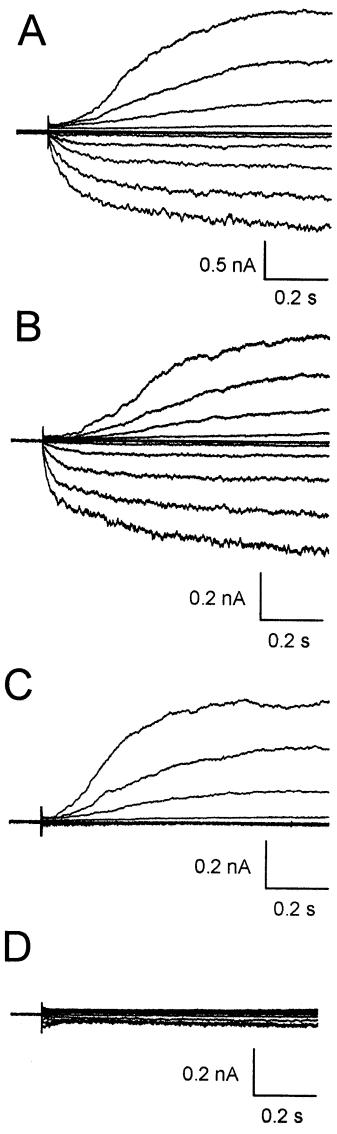

Fig. 3. Electrophysiological analyses of whole-cell K^+ currents in *A. thaliana* root hair protoplasts. Voltage- and time-dependent properties of inward and outward K^+ currents in root hair protoplasts from *Arabidopsis* wild-type (A), *skor1-1* (B) and the *akt1-1* knock-out mutants (C). In the presence of 10 mM Ba^{2+} in the bath, outward currents were completely blocked (D). In the whole-cell configuration voltage pulses were applied from a holding potential of −48 mV in 20 mV decrements in the range from +52 to −168 mV followed by a step to −88 mV. External solution contained 30 mM K-gluconate, 1 mM $CaCl_2$, 10 mM Mes/Tris (pH 5.6).

studies with intact *Arabidopsis* seedlings. 24–48 h following particle bombardment, GORK promoter-driven GFP fluorescence was detectable in guard cells (not shown) and root epidermal cells including root hairs (Fig. 2D,E). In addition GUS-staining of transgenic plants, expressing the *uidA* gene under control of the GORK promoter, confirmed our transient expression data (Fig. 2F,G). This indicates that two sensory epidermal cell types, guard cells and root hairs, differ in the composition and abundance of K^+ uptake channels (guard cells are dominated by KAT1 and KAT2 mRNA; (see [15] and [21])), but share the K^+ release channel GORK.

3.4. The root hair K^+ channels share basic functional properties with AKT1, ATKC1 and GORK

To provide evidence that the three *Shaker*-like K^+ channel genes encode dominant plasma membrane K^+ conductances in the root epidermis, we characterized the electrical properties of the root hair inward and outward rectifier. In contact with cellulases and pectinases the thin cell wall covering the growing tip of the root hair was removed and protoplasts released within 30 min (Fig. 2A,B). This root hair-enriched preparation, characterized by the expression of GORK, AKT1 and ATKC1 transcripts (Fig. 2C), was used for patch-clamp studies. As expected from the presence of AKT1 and ATKC1 mRNA, hyperpolarizing voltages elicited inward currents (Fig. 3A). In root hair protoplasts of the *akt1-1* mutant, however, the inward rectifier was absent (Fig. 3C). This finding is in agreement with the role of AKT1 as the dominant root K^+ uptake channel [5]. In contrast to *akt1-1*, *atkc1-1* plants did not lack the root hair inward rectifier. K^+ uptake currents of the latter, however, were characterized by pronounced alterations of the voltage-dependence, kinetics, selectivity and susceptibility towards regulatory cations ([22], unpublished). Since the residual K^+ channel properties resembled AKT1 when expressed in *Sf9* cells [23] and *atkc1-1* plants still express functional K^+ uptake channels, ATKC1 seems to represent a regulatory subunit of the root hair inward rectifier.

In line with the lack of *SKOR* transcripts in this cell type, K^+ currents in root hair protoplasts of the *skor1-1* mutant were wild-type-like, too (Fig. 3A,B). Thus the root hair outward rectifier is very likely to be encoded by either GORK or KCOs [24]. When in the whole-cell configuration of the patch-clamp technique protoplasts were clamped at −48 mV with 150 mM K^+ in the pipette and 30 mM K^+ in the bath, depolarizing pulses positive to −28 mV elicited voltage-dependent outward currents in wild-type, *skor1-1*, *kco1-1* ([25], unpublished) and *akt1-1* root hair cells (Fig. 3A–C). The voltage-dependence as well as the slow sigmoidal activation kinetics and potassium-dependence (see Fig. 3A and below) of this potassium conductance were in agreement with the properties of the delayed rectifier GORK expressed in *Xenopus* oocytes [15]. Since GORK represents a new root K^+ channel, this outward rectifier was characterized in detail.

Upon depolarization the root hair outward rectifier activated in a K^+-dependent manner. Relative open probabilities at different K^+ concentrations were well fitted with a Boltzmann function. Increase in external K^+ concentration caused changes in both activation threshold ($V_{1/2}$) and steepness (V_s) of the Boltzmann curve (Fig. 4A). Upon reduction of the external K^+ concentration from e.g. 90 to 10 mM, voltage activation curves shifted towards more positive potentials. This indicates that voltage-dependent gating of the root hair outward rectifier, just like GORK [15], is modulated in a potassium-dependent fashion. In agreement with the permeability of a K^+-selective channel, tail currents in response to a double-voltage pulse protocol reversed close to the Nernst potential for potassium (Fig. 4B). Under these conditions, a 56 mV shift in reversal potential per 10-fold change in external K^+ concentration was determined (Fig. 4C). When outside-out patches were excised from protoplasts characterized by pronounced outward K^+ currents (1 nA at +52 mV) time-dependent single-channel fluctuations were observed at posi-

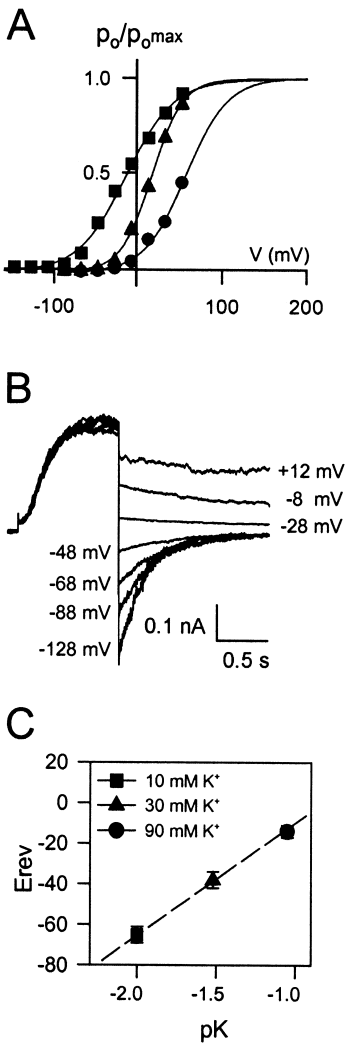

Fig. 4. Voltage-dependence and selectivity of the outward rectifier in *Arabidopsis* root hair protoplasts. A: Relative open probabilities of the outward rectifying K^+ channel as a function of voltage and K^+ concentration. Pulse protocol as in Fig. 2. External solution contained 10 (■), 30 (▲) or 90 mM K-gluconate (●). Open probabilities from three independent experiments were calculated as described in Section 2, normalized with respect to the maximum conductance and fitted by a Boltzmann function. B: Deactivation (tail currents) in response to a double-pulse protocol starting from a holding potential of −48 mV to a pre-pulse voltage of +52 mV and followed by voltage steps from +12 to −128 mV. Pipette and bath solutions contained 150 mM and 30 mM K-gluconate, respectively. C: Plot of reversal potential (E_{rev}) versus external K^+ concentration. E_{rev} was determined from tail currents measured in the presence of 10, 30 or 90 mM K-gluconate in the bath. The data points represent mean values ± S.E.M. ($n = 3$).

tive potentials (Fig. 5A,B). With 150 mM K-gluconate in the pipette and 30 mM K-gluconate in the bath, outward rectifying channels with a unitary conductance of about 12 pS reversed direction close to the calculated Nernst potential for potassium (Fig. 5C). In the presence of 1 mM of extracellular Cs^+, the inward rectifier was completely inhibited (not shown). In contrast, the K^+ efflux channel was unaffected even by 10 mM Cs^+. Upon application of Ba^{2+}, verapamil or TEA^+, however, the outward rectifier was blocked as described for GORK and the guard cell outward rectifier ([15] and data not shown).

3.5. Inactivation of the outward rectifier

The activation curves of the root hair K^+ release channel at different voltages were well fitted by a Hodgkin–Huxley-based model. Activation time constants (τ_{act}) were strongly dependent on voltage and varied from 230 ± 40 ms at −8 mV to 65 ± 30 ms at +112 mV. During prolonged voltage stimulation (10 s), macroscopic currents after reaching a peak slowly inactivated (Fig. 6A,B). The amplitude of inactivation increased with positive-going voltages from 5% at −8 mV to 60% at +112 mV. The steady-state inactivation current at voltages positive to +52 mV, however, remained constant (Fig. 6A,B). The inactivation time course was well fitted with a single exponential (not shown). Inactivation time constants (τ_{inact}) changed with applied voltage from 3.7 ± 0.8 s at −8 mV to 1.2 ± 0.5 s at +112 mV. The time-dependence of both activation and inactivation of the outward K^+ current was affected by the external K^+ concentration. When depolarizing pulses were applied from a holding potential of −48 mV to +52 mV, a 10-fold decrease in K^+ concentration strongly

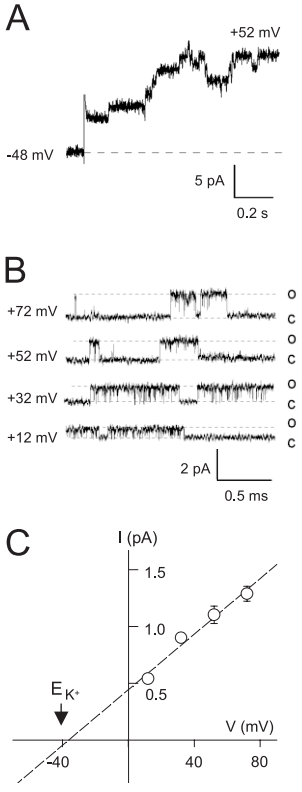

Fig. 5. Single-channel analysis of outward K^+ currents in *A. thaliana* root hair protoplasts. Outside-out patches were excised from protoplasts carrying macroscopic outward K^+ currents. A: Time-dependent openings of single channels were elicited by voltage step from −48 to +52 mV. B: Single-channel fluctuations in outside-out patches clamped to different steady-state voltages. C: Single-channel amplitudes plotted versus the membrane voltage. The slope conductance of the open channel amplitudes calculated from linear regression was 12 pS. The data represent mean channel amplitudes ± S.D. from three patches. Pipette solution contained 150 mM K-gluconate, 2 mM $MgCl_2$, 10 mM EGTA, 2 mM Mg-ATP, 10 mM HEPES/Tris (pH 7.4). Bath solution contained 30 mM K-gluconate, 20 mM $CaCl_2$, 10 mM Mes/Tris (pH 5.6).

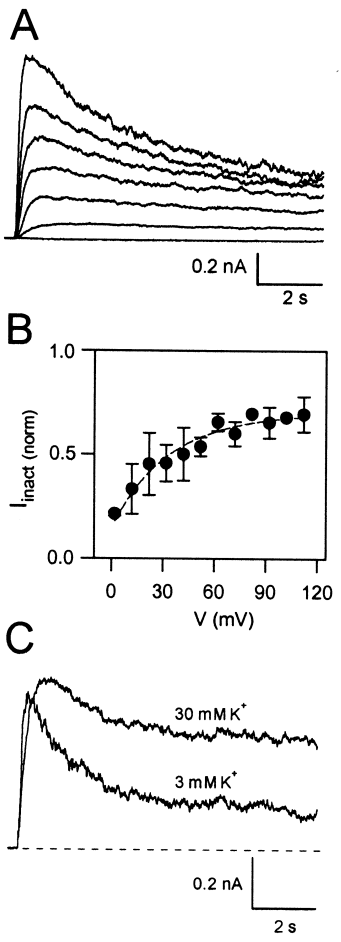

Fig. 6. Inactivation of outward rectifying K^+ channels in *A. thaliana* root hair protoplasts. A: Time and voltage-dependence of channel inactivation during prolonged (10 s) depolarizing pulses from a holding potential of −48 mV to +112 mV in 20 mV increments using standard solutions. B: Amplitude of current inactivation, normalized with respect to the maximal (peak) current and plotted as a function of voltage. The data points represent means ± S.E.M. of three independent experiments. C: Effect of 10-fold change in external K^+ concentration on time-dependent activation and inactivation of the outward rectifier. Currents were elicited by 8 s depolarizing voltage pulses from a holding potential of −48 mV to a test potential of +52 mV. External solution contained 3 or 30 mM K-gluconate, 1 mM $CaCl_2$, and 10 mM Mes/Tris (pH 5.6).

affected the kinetics of the outward rectifier (Fig. 6C). As observed with GORK expressed in *Xenopus* oocytes [15], a reduction in K^+ concentration in the bath from 30 mM to 3 mM accelerated the activation kinetics from sigmoidal to almost instantaneous. In addition, the amplitude of inactivation increased from 40 to 75% with a 2-fold decrease in the inactivation time constant τ_{inact} (from 2.8 ± 0.5 to 1.36 ± 0.7 s and Fig. 6C). In contrast, however, in *Xenopus* oocytes and HEK cells no inactivation was observed (not shown). This may indicate that the activation process represents a channel intrinsic property and that inactivation requires additional plant-derived factors. A similar phenomenon has been observed with mammalian RCK1 potassium channel α-subunits which inactivate only when co-expressed with auxiliary β-subunits [26]. Since the *Arabidopsis* genome also encodes a considerable number of β-subunit orthologues, future experiments will focus on proteins interacting with GORK which might restore the inactivation of this outward rectifier.

4. Discussion

During the life cycle of a plant, root cells face pronounced changes of their environment such as water and nutrient supply, mechanical barriers as well as the interaction with microorganisms [8]. Root hairs respond to these changes in their surroundings by immediate reactions, initiated by a depolarization of the membrane (see [13] for review). Experiments based on K^+ channel blockers indicated that outward rectifying K^+ channels might be responsible for depolarization-activated K^+ efflux and thus the control of membrane potential and turgor in general [27,28]. Turgor control in root hairs is pivotal since exocytotic processes continuously incorporate membrane and cell wall components into the fragile tip of these rapidly growing cells. Otherwise, solute concentrations in the soil, which dramatically change during drought and rainfalls, would affect the mechanical stability and hydration status of the root hairs. Fluctuations in the extracellular ionic composition at the surface of root hairs in response to elicitors or Nod factors are based on the influx of Ca^{2+} and efflux of K^+ and Cl^- (Fig. 1 and [1]).

4.1. K^+ channels repolarize the membrane potential

The properties of the inward and outward rectifying K^+ channels in root hair protoplasts are in agreement with the transient K^+ currents in intact root hairs resolved by extracellular ion-selective microelectrodes (Fig. 1, [13] for review). Following depolarization triggered by external stimuli, it is very likely that GORK prevents overshooting of the membrane potential by repolarization and thereby could contribute to the propagation of electrical signals. In line with its proposed role in electrical signalling this plant K^+ outward rectifier exhibits a pronounced potassium- and voltage-dependent inactivation (Fig. 6). Inactivation is a widespread phenomenon of animal voltage-gated cation channels in excitable membranes. In planta, however, reports on this phenomenon are rare. Inactivation has been described for the R-type anion channel ([7] for review) and the outward rectifier from guard cells [28]. In this study, we show that root hairs express GORK which together with SKOR represent the only *Shaker*-like outward rectifiers in the *Arabidopsis* genome. Similar to the guard cell anion channel and outward K^+ rectifier [28,29], the time course of inactivation in the root hair channel was well fitted by a single exponential with a time constant of several seconds. Inactivation of the root hair channel was voltage-dependent and modulated by changes in the external K^+ concentration (Fig. 6A–C). The latter feature of GORK may allow the cell to adjust the inactivation process to the degree of depolarization and the potassium gradient across the plasma membrane.

4.2. GORK seems to provide root hairs with a K^+ sensor

The outward rectifying K^+ channel from epidermal root cells shared the basic properties with the *Arabidopsis* guard cell K^+ release channel [28]. A common feature of these plant K^+ outward rectifiers as well as GORK and SKOR is their dependence of channel gating on the external K^+ concentration. An increase in K^+ concentration shifted the voltage gate of the root hair outward rectifier towards more positive po-

tentials (Fig. 4A). The outward rectifier thus provides a potassium sensor to the root hair when 'exploring' the nutrient sources in the soil. Apart from its proposed role in turgor control, signal transduction and K^+ sensing, GORK could be essential for the maintenance of root hair polarity. Model systems for studying plant cell polarity like root hairs, pollen tubes and *Fucus* eggs are characterized by an asymmetric distribution of sub-cellular structures and display distinct current patterns along these tip-growing cells [8,10,30,31]. Inward currents at the tip are carried by Ca^{2+}, H^+ and K^+, whereas outward currents at the base involve cation extrusion. Information on the polar localization is still lacking. Thus, future immuno-cytochemistry and patch-clamp on distinct root hair regions need to prove whether AKT1 and ATKC1 mediate K^+ influx at the tip and GORK K^+ efflux at the base.

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