THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 275, No. 50, Issue of December 15, pp. 39420–39426, 2000
© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

KDC1, a Novel Carrot Root Hair K^+ Channel

CLONING, CHARACTERIZATION, AND EXPRESSION IN MAMMALIAN CELLS*

Received for publication, April 7, 2000, and in revised form, September 1, 2000
Published, JBC Papers in Press, September 1, 2000, DOI 10.1074/jbc.M002962200

Patrick Downey‡§, Ildiko Szabò¶, Natalia Ivashikina‖, Alessandro Negro‡, Flavia Guzzo, Peter Ache‖, Rainer Hedrich‖, Mario Terzi‡¶, and Fiorella Lo Schiavo‡¶**

*From ‡Centro di Ricerca Interdipartimentale per le Biotechnologie Innovative, ¶Dipartimento di Biologia, Universita' di Padova, Via G. Colombo 3, 35121 Padova, Italy, the ‖Julius-von-Sachs-Institut für Biowissenschaften, Lehrstuhl für Molekulare Pflanzenphysiologie und Biophysik, Universität Würzburg, Julius-von-Sachs-Platz 2, 97082 Würzburg, Germany, and the **Istituto Policattedra, Universita' di Verona, Strada Le Grazie, Ca' Vignal 1, 37134 Verona, Italy*

Potassium is an essential nutrient which plays an important role in many aspects of plant growth and development. Plants have developed a number of highly specific mechanisms to take up potassium from the soil; these include the expression of K^+ transporters and potassium channels in root cells. Despite the fact that root epidermal and hair cells are in direct contact with the soil, the role of these tissues in K^+ uptake is not well understood. Here we report the molecular cloning and functional characterization of a novel potassium channel KDC1 which forms part of a new subfamily of plant K_{in} channels. *Kdc1* was isolated from carrot root RNA and *in situ* hybridization experiments show *Kdc1* to be highly expressed in root hair cells. Expressing the KDC1 protein in Chinese hamster ovary cells identified it as a voltage and pH-dependent inwardly rectifying potassium channel. An electrophysiological analysis of carrot root hair protoplasts confirmed the biophysical properties of the *Kdc1* gene product (KDC1) in the heterologous expression system. KDC1 thus represents a major K^+ uptake channel in carrot root hair cells.

Potassium is one of the most abundant inorganic cations in higher plant cells. This macronutrient is involved in maintaining electro-neutrality and is an essential co-factor for a number of enzymes; especially those involved in respiration and photosynthesis (1). Potassium channels play an important role in K^+ uptake as well as the control of membrane potential (2), growth, and turgor driven movements (3, 4).

Potassium channels can be divided into outward rectifiers (K_{out}) which excrete potassium from the cell, inward rectifiers (K_{in}) which transport potassium ions into the cell, and largely voltage-independent channels ($K_{in/out}$) which are able to catalyze both processes. The first genes encoding plant K^+ channels were isolated by the complementation of yeast mutants deficient in potassium uptake (5, 6). Recently other genes have been isolated by library screening with heterologous, oligonucleotide, and EST probes (7–11). Most of the plant K^+ channels isolated so far bear a striking resemblance to the *Shaker* family of potassium channels, showing the highest homology with members of the *eag* gene family which can be either inward or outward rectifying (12, 13). Plant homologues segregate into four subfamilies SKOR1(K_{out}), AKT2/3(K_{in}), KAT1(K_{in}), and AKT1(K_{in}). The SKOR1 channel is expressed in cells surrounding root xylem vessels (10), AKT2/3 in the phloem of stem and leaves (14), KAT1-like channels in guard cells and flowers (7, 15), AKT1 mainly in the cortex and endoderm tissues of the root (16, 17).

The present work was directed toward the isolation of K^+ channels expressed in root hair cells. Using a PCR[1] amplification strategy with degenerate oligonucleotides we cloned an inwardly rectifying K^+ channel KDC1 from carrot root RNA. A combination of *in situ* hybridization experiments and comparative electrophysiological studies of the gene product expressed in CHO cells and K^+ channels in root hair cells identified KDC1 as the major inwardly rectifying K^+ channel of carrot root hair cell plasma membranes.

EXPERIMENTAL PROCEDURES

Plants, Bacterial Strains, and Growth Conditions—Carrot plants *Daucus carota* L. cv. S. Valery were grown in a greenhouse in soil with 16-h light 22 °C and 8-h dark 15 °C periods. Plants were sown at a density of 20 plants/15-cm pot and watered daily. The *Escherichia coli* strains XL_2Blue (Stratagene) and DH5α (Life Technologies, Inc., Gaithersburg, MD) were used as hosts for plasmid DNA using standard methods (18).

Isolation of Kdc1 from Carrot Root RNA—Degenerate primers based on the highly conserved potassium channel pore region were designed and used in an reverse transcriptase-PCR strategy. Total RNA was isolated from the roots of newly germinated carrot seeds which are highly enriched for root hairs, 5 μg were used to prepare cDNA using the degenerate reverse primer in place of the standard poly(dT) primer. In PCR reactions 150 pM of each degenerate primer were used, dNTPs were used at 200 μM and $MgCl_2$ at 1.5 mM, 2.5 units of AmpliTaq polymerase (PerkinElmer Life Sciences) were used. The reaction conditions were as follows, 30 s at 94 °C, 1 min at 37 °C, 1 min at 72 °C (5 cycles), followed by 30 s at 94 °C, 1 min at 48 °C, and 1 min at 72 °C (35 cycles). Several sequences showing homology with known potassium channel coding genes were amplified, one of these sequences *Kdc1* was chosen for further study. The 5′ and 3′ extremities of *kdc1* were identified by rapid amplification of cDNA ends PCR, using gene specific primers. The complete coding sequence was isolated by standard PCR (30 cycles) using a proofreading polymerase PFU Turbo™ (Stratagene), three independently isolated clones were subcloned and sequenced, no sequence discrepancies were found. The amplified *Kdc1* coding sequence was subcloned into pCDNA3 using standard procedure (18). The

* This work was supported by the Biotechnology Program of the European community, grant BIO 4CT 960689 (to M. T.), "Biotecnologie Vegetali" Ministero delle Politiche Agricole and P.F. Biotecnologia C.N.R. (to F. L. S.). 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.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ249962.

§ To whom correspondence should be addressed: Centro di Ricerca Interdipartimentale per le Biotecnologie Innovative, C.R.I.B.I Biotechnology Centre, Viale G. Colombo 3, 35121 Padova, Italy. Tel.: 39-049-8276163; Fax: 39-049-8276159; E-mail: pdowney@civ.bio.unipd.it.

[1] The abbreviations used are: PCR, polymerase chain reaction; CHO, Chinese hamster ovary; Mes, 4-morpholineethanesulfonic acid; GFP, green fluorescent protein; ER, endoplasmic reticulum.

This paper is available on line at http://www.jbc.org

Downloaded from http://www.jbc.org/ by guest on July 24, 2018

Kdc1 stop codon TGA was changed to TCA by PCR and the modified sequence was cloned into plasmid pEGFP (CLONTECH) by PCR to create an in-frame fusion with the GFP. All constructs were confirmed by DNA sequencing using the T7 sequencing kit (Amersham Pharmacia Biotech). Sequencing primers were obtained from Primm srl (Milan, Italy). Sequence data analysis was performed using Lasergene software (DNASTAR Inc). Protein sequence alignments were performed using standard algorithms (19, 20). The *E. coli* strains XL_2Blue (Stratagene) and DH5α (Life Technologies, Inc., Gaithersburg, MD) were used as hosts for plasmid DNA.

Northern Blotting—Total RNA was extracted from various plant tissues by the guanidine hydrochloride method (21). After denaturation in 40% formaldehyde, RNA (15 μg) was separated on 1.2% agarose gels containing 15% formaldehyde and blotted onto Hybond N nylon membranes (Amersham Pharmacia Biotech, Little Chalfort, UK) with 20 × SSC. Prehybridization and hybridization were performed at 42 °C in 50% formamide, 5 × SSPE, 1% Sarkosyl, 10% dextran sulfate, and 100 μg/ml denatured salmon sperm DNA. Filters were washed for 30 min each in 1 × SSC, 0.5% SDS and 0.2 × SSC, 0.5% SDS at 60 °C, autoradiography was performed between intensifying screens at −70 °C for 2 days on Kodak X-Omat AR film.

Whole Mount Hybridization—Carrot root segments were embedded in Paraplast as described previously (22). Eight-μm thick sections were placed on poly-L-lysine (Sigma)-coated slides. The Paraplast was removed and slides were hydrated. Samples were treated with 1 μg/ml Proteinase K (Roche Molecular Biochemicals), ethanol dehydrated, and air-dried. Hybridizations were performed for 16 h at 42 °C, using 200 ng/ml digoxigenin-labeled sense or antisense riboprobes (Roche Molecular Biochemicals). After washing, slides were treated with 25 μg/ml RNase A (Fluka) incubated 1 h at room temperature with anti-digoxigenin-alkaline phosphatase conjugate (Roche Molecular Biochemicals) and extensively washed with phosphate-buffered saline. Samples were stained for 16 h at room temperature using Fast Red tablets (Roche Molecular Biochemicals) as substrate. Localization was documented using a Leica DMRB microscope.

Cell Culture and Transfection—CHO-KI cells were cultured according to standard conditions and transfected using LipofectAMINE™. Transfected cell cultures were incubated in normal medium and maintained at 30 or 37 °C in a humidified incubator in the presence of 5% CO_2. Transfected CHO cells used in electrophysiological analyses were incubated at 30 °C. All reagents were obtained from Life Technologies, Inc., Gaithersburg, MD. GFP fluorescence was examined using an inverted fluorescence Olympus TM20 microscope. Images were recorded with a 1000 × 800 digital CCD camera (Princeton Instruments) and processed using Adobe PhotoShop 5.0 Software. The fluorescence filter set was obtained from the Chroma Technology Corp., excitation HQ480/40, dichroic Q480LP, and emission HQ510LP.

Patch Clamp Analysis—For whole cell experiments with CHO cells the bath solution contained 150 mM potassium gluconate, 20 mM KCl, 1 mM $CaCl_2$, 2.5 mM $MgCl_2$, and 10 mM Hepes, pH 6.8. The pipette solution was 114 mM potassium gluconate, 20 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, 10 mM Hepes, pH 7.35, and 4 mM ATP. For selectivity experiments the solution was as above, but potassium gluconate was substituted with 134 mM KCl, while the bath solution contained 50 mM K gluconate and 120 mM NaCl. Root hair protoplasts were obtained essentially as described by Gassmann and Schroeder (23). Isolated protoplasts were washed twice in the bath solution, containing 1 mM $CaCl_2$ (osmolarity 280 mosmol kg^{-1}, pH 5.6) and filtered through a 50-μm nylon mesh. Protoplast preparations were kept on ice for up to 4 h. In all measurements the standard external solutions contained: 10 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. The pH of the external solutions was adjusted with 10 mM citric acid/Tris, pH 4.5, Mes/Tris, pH 5.6, and Hepes/Tris, pH 7.0. Modifications in solute compositions are shown in the figure legends. The 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. Patch pipettes were prepared from Kimax-51 glass capillaries (Kimble products, Vineland, NY) and coated with silicone (Sylgard 184 silicone elastomer kit, Dow Corning). The pipette resistance in the standard solution was 3–4 MΩ in experiments with CHO cells and 6–7 MΩ in experiments with root protoplasts. Measurements were performed in the whole cell and outside-out mode (24) 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), stored on hard disc, and analyzed using software PULSE and PULSEFIT (HEKA Elektronik, 1999) and IGORPro (WaveMetrics Inc., 1999). The command voltages were corrected off-line for liquid junction potentials as described previously (25).

RESULTS

Isolation of Kdc1 from Root cDNA—Kdc1 was isolated by reverse transcriptase-PCR from carrot root mRNA. The predicted protein sequence of the *Kdc1* gene contains 629 amino acids with a calculated molecular mass of 72,277 daltons. An hydropathy analysis identified six segments which exceed the threshold value of 20 Kcal/mol (Fig. 1*A*) which correlates with stable insertion of a peptide into the lipid bilayer (26). The potassium channel signature sequence of the KDC1 protein is TLTTTGYGD which in common with other plant K^+ channels is slightly modified compared with the classical sequence TMTTVGYGD (27). Taken together these data suggest that KDC1 has a membrane topography similar to *Shaker*-like channels with six membrane spanning segments and a translocating pore positioned between the last pair of helices. The predicted cyclic nucleotide-binding site can be found between amino acid residues 379 and 498. There are several potential sites for phosphorylation by cAMP kinase ((RK)(2)-*X*-(S/T)) (28), protein kinase C ((ST)-*X*-[RK]) (29), casein kinase II ((ST)-*X*-(DE)) (30), and tyrosine kinase ((RK)-*X*-(2,3)-(DE)-*X*-(2,3)-Y) (31) (see Fig. 1*B*).

The KDC1 protein is not very similar to the $K(_{out})$ channel SKOR1 (38% identity in the transmembrane domain and approximately 20% overall). However, an alignment of the KDC1 protein sequence with the predicted protein sequences of members of the AKT2/3, KAT1, and AKT1 subfamilies shows a reasonably high degree of amino acid conservation in the transmembrane domains of the proteins. KDC1 shares 53% amino acid identity with AKT2/3, 55% amino acid identity with KAT1, and 59% with AKT1 (amino acid residues were aligned from Val^{67} to Val^{302}) see Fig. 1*C*, the overall amino acid identities are, however, low, less than 40%. KDC1 is, however, highly homologous to the putative *Arabidopsis* potassium channel ATKC1 (GenBank™ ATU81239). The homology is not restricted to the N-terminal transmembrane domains. KDC1 and ATKC1 show an overall identity of 60% and on the basis of sequence homology appear to form a new subgroup within the increasing plant K^+ channel gene family (Fig. 1*D*). KDC1 and its putative *Arabidopsis* counterpart ATKC1 are the smallest K^+ channels of the plant shaker K^+ channel family which have been isolated so far. These proteins contain the classical transmembrane domain, the junction linking it to the putative cyclic nucleotide-binding site and the conserved C-terminal sequence (see Fig. 1*B*). The highly conserved C-terminal sequence is enriched for hydrophobic and acidic amino acids and has thus been denominated (K_{HA}). This region has been shown to be involved in the interaction of different α subunits in two-hybrid experiments (32) and is thought to be involved in the tetramerization process (32, 33). The conserved sequence motifs found in the KDC1 and ATKC1 channels are common to all *shaker*-like plant K^+ channels isolated so far. KDC1 and ATKC1 do not contain the ankyrin-like repeat regions which are common to channels of the AKT1 and AKT2/3 subfamilies. The role played by these ankyrin-like regions in plant K^+ channels remains to be determined, the ankyrin repeat is one of the most common protein sequence motifs and often plays a role in protein-protein interactions (34).

All plant inwardly rectifying potassium channels isolated so far including the putative ATKC1 channel contain a histidine residue in the potassium selectivity filter. This histidine residue is found three residues downstream of the last G of the sequence GYG, and has been shown to be involved in pH sensing in the KST1 channel (35). The pore histidine is apparently not a universal pH sensor as mutations in the equivalent

Downloaded from http://www.jbc.org/ by guest on July 24, 2018

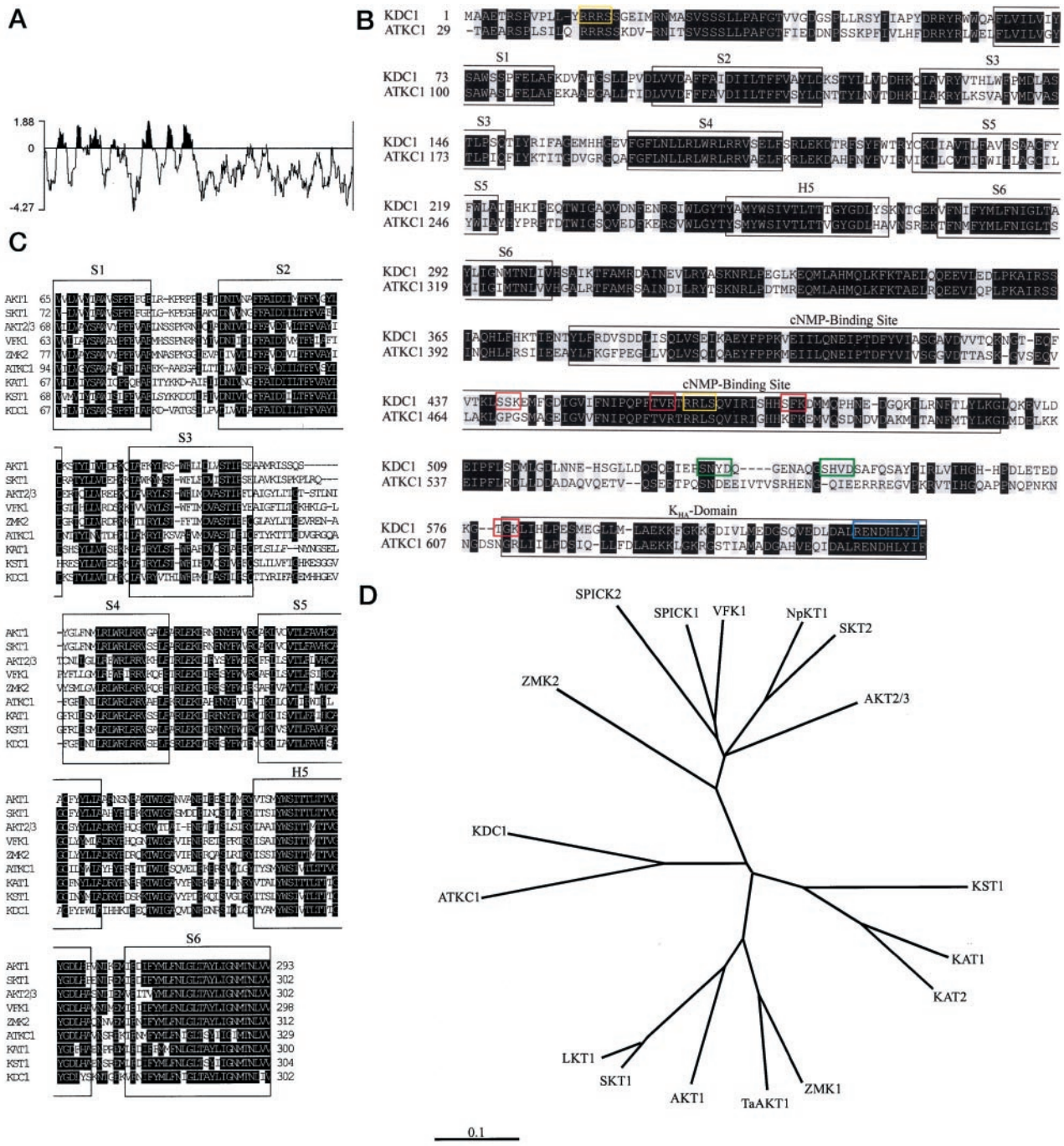

FIG. 1. **Analysis and comparison of KDC1.** *A*, an hydropathy analysis of the deduced amino acid sequence of KDC1. The plot identifies Goldman-Engelman-Steiz potential transbilayer helices (marked in *black*) using a window of 20 amino acids. *B*, an alignment of the predicted amino acid sequence of KDC1 with ATKC1. The transmembrane zones S1-S6 are *boxed* as are the putative cyclic nucleotide-binding sites and K_{HA} domains. Sites of possible phosphorylation of the KDC1 protein are indicated by *colored boxes* (see main text). The KDC1 and ATKC1 (GenBank™ ATU81239) sequences were aligned using the algorithm of Lipman and Pearson (19). Identical residues are *boxed* in *black*, while highly similar residues are *boxed* in *gray*. *C*, an alignment of various plant potassium channel sequences, the sequences were aligned from the valine residue at the start of the S1 region up to the valine at the end of the S6 region (see main text). A clustal algorithm (20) was used to align the sequences. A consensus sequence was arbitrarily chosen when 5 of the 9 sequences contained an identical residue, residues which match the consensus are in reverse lettering, *i.e. white lettering* on a *black background*. (GenBank™ accession numbers: AKT1 (X62907), SKT1 (T07651), AKT3 (U44745), VFK1 (Y10579), ZMK2 (AJ132686), ATKC1 (U81239), KAT1 (X93022), KST1 (X79779), and KDC1 (AJ249962). *D*, phylogenetic tree of all of the available plant potassium channels (sequences not previously aligned have the following GenBank™ accession numbers (KAT2 (CAA16801), LKT1 (X96390), NpKt1 (AB032074), ZMK1 (Y07632), SKT2 (Y09699), SPICK1 (AF099095), SPICK2 (AF145272), and TaAKT1 (AF207745). The entire peptide sequences were aligned using the clustal algorithm (20) and the dendrogram was constructed using TreeView.

residue in the KAT1 channel did not led to changes in pH sensitivity (36). In the KDC1 channel, the pore histidine is replaced by a tyrosine residue, indicating that the highly conserved "pore histidine" is not ubiquitous among plant K^+ channels.

Expression of Kdc1 in Planta—To determine the expression pattern of *Kdc1*, RNA was isolated from the leaf, root, stem, and tubers of greenhouse grown carrot plants and Northern blot analyses were performed using the *Kdc1* cDNA as a radioactively labeled probe. The *Kdc1* cDNA detected an approximately 2.1-kilobase mRNA in root tissue, no expression was seen in leaves, stem, or tubers (Fig. 2*A*). In order to define the

Downloaded from http://www.jbc.org/ by guest on July 24, 2018

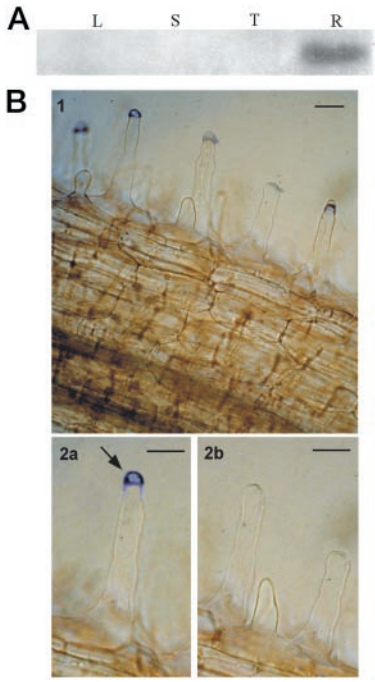

FIG. 2. **Expression of *kdc1* in carrot tissues.** *A*, Northern blot analysis, total RNA was extracted from the leaf (*L*), stem (*S*), tuber (*T*), and root (*R*) of 10-week-old greenhouse grown carrot plants, 15 μg of total RNA were loaded per lane. After transfer to a nylon membrane, hybridization was performed using the *Kdc1* cDNA as a probe. A 1-kilobase RNA marker was used to size the *Kdc1* mRNA. *B*, whole mount hybridization performed on carrot roots, *1*) *Kdc1* expression is evident in root hair cells (antisense transcript); *2a*) enlargement of a single root hair (antisense transcript), the *arrow* indicates that expression is localized to the tip of root hair cells; *2b*) no signal is present when the sense transcript (negative control) is used. *Bars,* 50 μm.

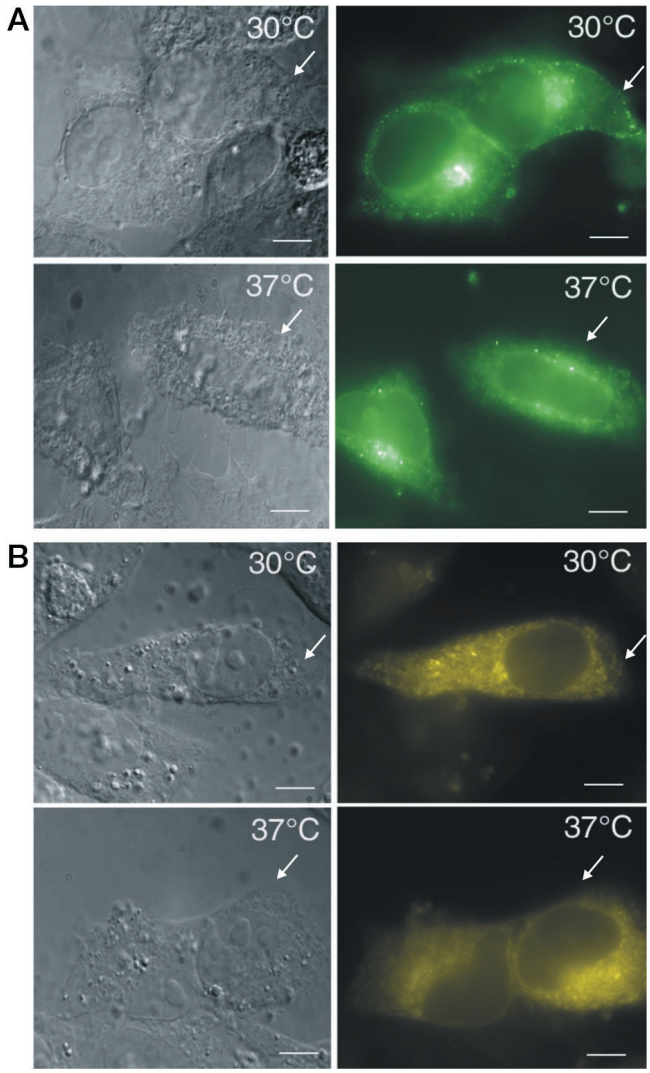

FIG. 3. **The effect of temperature on the cellular localization of the KDC1::GFP fusion protein.** CHO cells were transfected with the KDC1::GFP fusion protein (*A*) or an ER-targeted YFP (pEYFP-ER CLONTECH) (*B*) and incubated at 30 or 37 °C. The cells were then fixed and examined using differential interference contrast microscopy (*left*) and fluorescence microscopy (*right*). *A*, at 30 °C the KDC1::GFP fusion protein is well localized at the ER and green fluorescence can be seen throughout the cell and close to or at the plasma membrane, while at 37 °C the protein clearly remains blocked at the ER. *B*, the yellow fluorescent ER targeted protein is clearly localized at the ER regardless of the incubation temperature. Plasma membranes are indicated with an *arrow*. *Bars*, 10 μm.

cell types expressing KDC1 we performed whole mount hybridization experiments on carrot roots, these were hybridized with either the antisense *Kdc1* RNA (to detect *Kdc1* mRNA) or the sense transcript (as a negative control). Specific signals were only detected in the presence of the antisense RNA probe. These experiments showed that *Kdc1* expression is localized to the root epidermal cells with expression being particularly evident in root hair cells (Fig. 2*B*).

Analysis of a KDC1-GFP Fusion Protein—To follow the fate of the KDC1 protein in animal cells, we created a fusion protein between KDC1 and the green fluorescent protein (GFP) and followed its progress in transfected CHO cells. When transfected cells were incubated at 37 °C, the protein remained largely blocked at the endoplasmic reticulum and did not apparently proceed to the plasma membrane. When transfected cells were grown at 30 °C, the fusion protein proceeded beyond the ER and could be detected close to or at the plasma membrane (Fig. 3*A*). The reduction in incubation temperature did not influence the localization of an ER-tagged YFP (Fig. 3*B*). As localization experiments showed that the KDC1 protein was more efficiently targeted to the membrane at 30 °C, *Kdc1* transfected CHO cells were incubated at 30 °C before being used in electrophysiological experiments.

Whole Cell Recordings of Kdc1-transfected CHO Cells—Control (pCDNA3 transfected) CHO cells did not show channel activity ($n = 12$). Transient expression of KDC1, however, resulted in the appearance of a voltage-dependent inwardly rectifying channel in approximately 30% of transfected cells ($n = 79$) (Fig. 4*A*) 2 to 4 days after transfection. Fig. 4*B* displays the current-voltage relationships of the channels recorded with 10, 50, and 170 mM external potassium. The intrinsic voltage dependence and selectivity of the KDC1 channel were determined by normalization and concentration-dependence analysis (Fig. 4, *B* and *inset*), respectively. Under asymmetric ionic conditions the Nernst potential for Cl^- ($E_{cl}-$) was −2.8 mV, and E_{k^+} was −25.5 mV. The current reversal potential for KDC1, as determined from the I-V curves was −23 ± 3.8 mV ($n = 5$), indicating a high selectivity for potassium over chloride and sodium ions ($P_{Na}/P_K = 0.04$). The half-time of current activation was 28 ms ± 2.7 at −160 mV ($n = 4$) and 11 ± 1.5 ms at −180 mV ($n = 8$). The addition of 5 mM of the K^+ channel blocker Cs^+ reduced the current by 31 ± 8% ($n = 5$), while 10 mM completely blocked channel activity ($n = 5$) (Fig. 4*C*). When applying 10 mM tetraethylammonium, another K^+ channel blocker, the whole cell current recorded at −180 mV was inhibited by 40 ± 3.7% ($n = 5$). When the pH was decreased from pH 7.0 to 5.9 there was a 1.8-fold increase in the steady state current recorded at −160 mV ($n = 5$) (Fig. 4*D*). In the absence of ATP in the pipette solution, there was a rapid rundown of the KDC1 induced current (approx-

Downloaded from http://www.jbc.org/ by guest on July 24, 2018

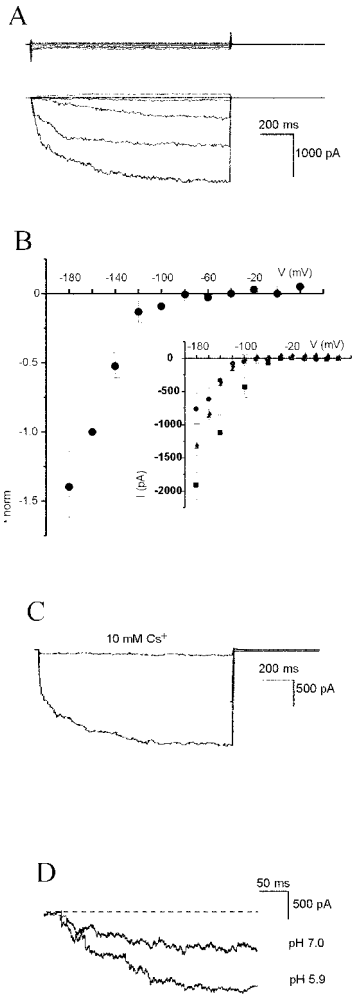

FIG. 4. **KDC1 expressed in CHO cells gives rise to an inwardly rectifying current.** Representative whole cell currents recorded from a control CHO cell and a *Kdc1*-transfected CHO cell (*A*). The holding potential was 0 mV. Pulses of 600 ms duration were applied in 40 mV steps at 45-s intervals, ranging from −180 mV to +60 mV, currents were recorded in the presence of the 170 mM external potassium medium (sampling frequency 2 kHz, filtering 1 kHz). *B*, dependence of KDC1 channel currents on external K^+ concentration. Intrinsic voltage-dependence of KDC1 in the presence of 10 mM external potassium. *Inset,* steady state current-voltage curves of whole cell recordings in the presence of 10 mM (●) (n = 5), 50 mM (▲) (n = 4), and 170 mM (■) (n = 18) external potassium, respectively. *C*, whole cell traces recorded from a *Kdc1*-transfected cell before and after the addition of 10 mM CsCl (V_h = −180 mV). *D*, whole cell traces recorded from a *Kdc1* transfected cell at pH 7.0 and 5.9.

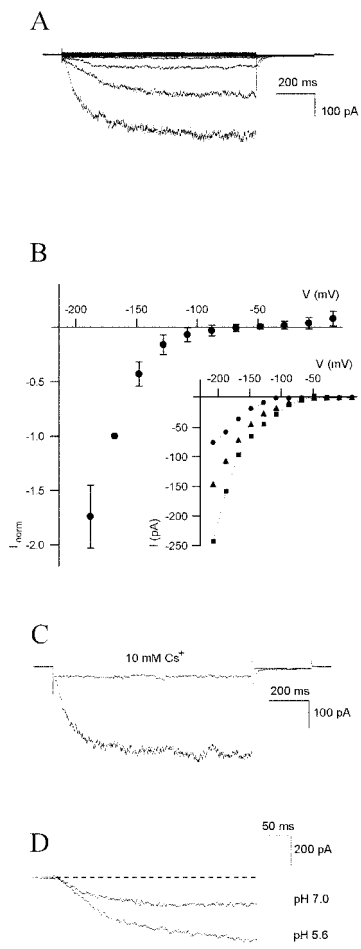

FIG. 5. **Voltage and time-dependent properties of whole cell inward K^+-currents in root hair protoplasts.** *A,* representative macroscopic currents obtained by changing the membrane voltage from a holding potential of −40 mV in 20-mV decrements from +20 to −180 mV, followed by a pulse to −80 mV (150 mM K^+ in the pipette, 10 mM K^+ in bath). *B*, current voltage characteristics of normalized steady-state inward currents at 10 mM K^+ in bath solution (mean ± S.D., n = 7). *Inset,* steady state current-voltage curves of whole cell recordings in the presence of 10 (●), 20 (▲), and 30 mM (■) external K^+, respectively. *C*, block of inward current by external Cs^+. *Traces* show currents at voltage pulse −180 mV from holding potential −40 mV and followed by a pulse to −80 mV, measured before and after the addition of 10 mM CsCl. *D*, pH dependence of macroscopic current. Whole cell traces were recorded at pH 7.0 and pH 5.6.

imately 50%) within 5 min (n = 5).

Whole Cell Recordings of Root Hair Protoplasts—In experiments with carrot root hair protoplasts 7 out of 11 cells displayed inward currents (Fig. 5*A*). In at least half of these patches, an additional instantaneous voltage-independent component was also observed, which we attribute to the presence of a non-selective cation channel (data not show). Thus, we focused on the analysis of the time-dependent inward currents. Whole cell configurations of protoplasts were clamped at −40 mV with 150 mM K^+ in the pipette and 10 mM K^+ in the bath. Hyperpolarizing pulses negative to around −120 mV activated inward currents (Fig. 5*B*), which, according to tail analysis, reversed close to the Nernst potential for K^+ (−69 mV). To determine the cation selectivity of the inwardly rectifying channel, the external K^+-gluconate concentration was changed from 10 to 20 and 30 mM (Fig. 5*B, inset*). An increase in the steady-state current amplitude and a positive shift in E_{rev} (10–15 mV per 10 mM K^+) identified this inwardly rectifying channel as K^+-selective. When potassium was substituted with Na^+, a relative permeability of P_{Na}/P_K = 0.07 was determined, indicating a high selectivity for K^+ over Na^+. In addition, the effect of the extracellular K^+-channel blockers Ca^{2+}, Ba^{2+}, and Cs^+ on the inward K^+ current was examined. Addition of 20 mM Ca^{2+} resulted in a voltage-dependent block with a 30–32% decrease in the steady-state current amplitude at −180 mV (data not shown). The external application of 5 mM Cs^+ resulted in a 27 to 29% block of the steady-state current, while 10 mM Cs^+ blocked up to 94% of the current at −180 mV (Fig. 1*C*). The addition of 5 mM Ba^{2+} to the extracellular solution completely blocked the channel (data not shown). Changes in the external pH showed that the inward K^+ current is activated by protons (Fig. 1*D*). When the pH was changed from 5.6 to 4.5, the steady-state current amplitude increased 1.8-fold and the voltage dependence was shifted to more positive potentials. Increasing the pH to 7.0 resulted in a 2-fold decrease in K^+ channel current with a shift in the activation potential to more negative values.

Downloaded from http://www.jbc.org/ by guest on July 24, 2018

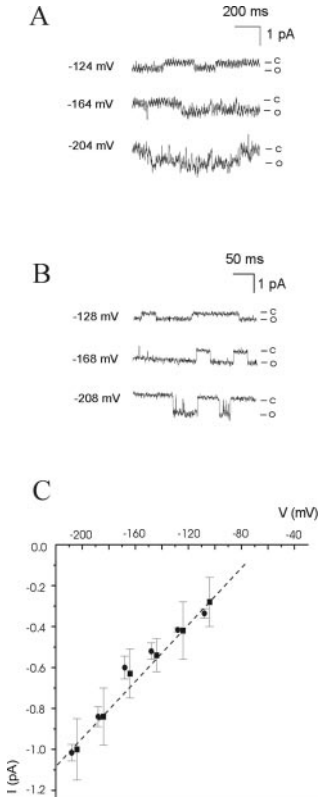

FIG. 6. **Single channel activity in root hair protoplasts and KDC1-transfected CHO cells.** Channel activities recorded from a KDC1-transfected CHO cell (*A*) and a root hair protoplast (*B*) in outside-out excised patches (bath 10 mM K^+, pipette 150 mM K^+). *C*, current-voltage relationship of the above channel activities from root hair protoplasts (●) and KDC1-transfected CHO cells (■).

Single Channels—To examine the single channel conductance, measurements were performed in the outside-out configuration. With 150 mM K^+ in the pipette and 10 mM K^+ in the bath solution, single channel activity was observed upon hyperpolarizing the membrane to voltages more negative than −100 mV in both KDC1-transfected CHO cells (Fig. 6*A*) and root hair protoplasts (Fig. 6*B*). The current voltage relationship revealed conductances in the 7–10 pS range in both cases, with an apparent reversal potential close to the theoretical E_{rev} for K^+ (Fig. 6*C*). Importantly, such activity was observed in KDC1-transfected cells but was never seen in nontransfected cells.

DISCUSSION

Most of the work performed to date on the characterization of plant potassium channels has focused on the model plant *Arabidopsis thaliana*. A molecular and electrophysiological analysis of K^+ channels from other plant species, particularly crop species, is needed before general conclusions about the roles of K^+ channels in plant growth and development can be drawn.

Here we describe the molecular cloning and functional analysis of an inwardly rectifying K^+ channel KDC1 from carrot. Northern blotting demonstrated that *Kdc1* is mainly expressed in the root and whole mount hybridization showed it to be localized at the tip of root hair cells. KDC1 is remarkably similar to the putative *Arabidopsis* K^+ channel ATKC1 (GenBank™ ATU81239). ATKC1 (which has not yet been characterized at the electrophysiological level) is also predominantly expressed in root tissue and has a similar expression pattern to KDC1.[2] The KDC1 and ATKC1 proteins share 60% amino acid identity. These channels are expressed in root tissue but do not contain the ankyrin-like repeats which are characteristic of proteins of the AKT1 family. Based on their similar expression patterns and peptide sequences we suggest that these two proteins represent a new subfamily of plant K^+ channels. Plant $K_{(in)}$ channels have previously been divided into two subgroups based on the presence (AKT1-like) or absence (KAT1-like) of the ankyrin-like repeat region. A recent detailed electrophysiological analysis of the AKT2/3 channel showed it to be only very weakly rectifying and blocked by protons (14) thus it is clearly different to KAT1- or AKT1-like channels which are strongly rectifying and activated by acid pH. We propose that plant K_{in} channels can in fact be divided into four subfamilies. KAT1-type channels which are expressed in guard cells and flowers, do not contain ankyrin-like repeats and are activated at acidic pH values (7, 15), AKT1-like channels are mainly expressed in root tissue are activated at acidic pH values and contain ankyrin-like repeats (9, 16, 17), AKT2/3-like channels are expressed in vascular tissue contain ankyrin-like repeats and are blocked by acidic pH (14, 37),[3] and KDC1-like channels which are mainly expressed in root epidermal tissues are activated at acidic pH values and do not contain ankyrin-like repeat regions. When an alignment of the predicted amino acid sequences of these channels is performed using either the transmembrane domains or the entire peptide sequences the channels divide into these groups. As new channels are discovered and detailed expression analyses performed these groupings may of course change, but they appear to reasonably represent the sequence and expression data presently available.

KDC1, like ATKC1,[4] did not express in *Xenopus* oocytes and was unable to complement the CY162 yeast mutant (38). Sf9 cells have been used to study plant K_{in} channels which could not be expressed in *Xenopus* oocytes, however, as only certain batches of these cells are able to withstand hyperpolarizations beyond −150 mV (39) they are clearly not ideal for analyzing plant K^+ channels. CHO cells have been widely used to express mammalian proteins and this cell line represents a preferred system for exogenous K^+ channel expression (40). To follow the fate of KDC1 in CHO cells we fused the KDC1 protein with the GFP and performed localization experiments. A number of mammalian ion channels have been successfully tagged with GFP and the presence of the tag allowed localization studies to be performed (41, 42). Under standard culture conditions both the KDC1::GFP fusion and a KAT::GFP protein (data not shown) were blocked at the ER. Reducing the incubation temperature to 30 °C helped to ensure functional insertion into the plasma membrane, similar results have been reported for a misfolded CFTR channel protein (43). As 37 °C is not a physiological temperature for plant cells, plant proteins may not fold correctly at such an elevated temperature, reducing the temperature could help the proteins to fold more correctly and thus reach the plasma membrane. We have also recently expressed the KAT1 protein in CHO cells, confirming the validity of the system. CHO cells represent a robust system for expressing plant K^+ channels and we therefore think that they could become the system of choice for expressing plant K^+ channels which do not express in *Xenopus* oocytes.

The whole cell electrophysiological analysis of KDC1 in CHO cells showed it to be an inwardly rectifying potassium specific channel. KDC1 is blocked by cesium and tetraethylammonium which are specific inhibitors of K^+ channels, and showed a low

[2] K. Palme, personal communication.
[3] P. Ache, submitted for publication.
[4] K. Palme, personal communication.

Downloaded from http://www.jbc.org/ by guest on July 24, 2018

permeability to sodium. Potassium and sodium are the only physiologically relevant alkaline metals that root hairs are exposed to and KDC1 is clearly selective for potassium over sodium. Interestingly, the KDC1 channel is strongly activated at acidic pH values and there was a large increase in current when the pH of the bath solution was reduced from pH 7.0 to 6.0. The pH in root cell walls is acidic. Measurements in corn root tissues showed that the apoplastic pH ranges 5.1–5.6 in the cortex (44) and 4.5–4.9 in the epidermis (45). CHO cells are unable to tolerate such acidic pH. A whole cell analysis of carrot root hair protoplasts showed that the predominating conductance was a potassium-specific current, confirming the findings of Gassmann and Schroeder (23) with wheat root hair cells. Our whole cell results showed that carrot root hair cells contain an inwardly rectifying K^+ current which is blocked by cesium and is much less permeable to sodium. We were able to measure the current at pH values ranging from pH 7 to 4.5, the current was clearly increased as the pH of the external solution reduced, showing that the K^+ current is acid activated. Similar findings have been recently reported with barley root protoplasts (46). The biophysical and pharmacological characteristics of the KDC1 channel in CHO cells recorded in the whole cell configuration were in good agreement with those obtained from root hair protoplasts (were *Kdc1* is highly expressed). Single channel analyses further suggest that KDC1 underlies the K^+ channel activity observed in root hair protoplasts.

While our data suggest that KDC1 is the main conductance in the root hair plasma membrane this does not exclude the possibility that other K_{in} channels may also contribute to the whole cell activity. For example, in transgenic *Arabidopsis* plants transformed with an AKT1 promoter-GUS fusion, GUS activity was mainly detected in the root endoderm and cortex but was also shown to be present in root hair cells (16). Very recently, a new tomato channel LKT1, a member of the AKT1 family, has also been shown to be expressed in root hair cells (47). Thus it is likely that channels from both the AKT1 and KDC1 families are in fact present in root hair cells.

Root hair cells are tip growing tubular outgrowths of epidermal cells which serve to increase surface area and penetrate between soil particles to provide access to nutrients in undepleted soil regions (48). The localization of KDC1 in root hair cells and its biophysical characterization suggest that this channel is very probably involved in potassium uptake from the soil. We cannot, however, exclude the possibility that KDC1 is not directly involved in K^+ uptake, but plays a role in the growth and/or development of root hairs. However, the fact that we always saw *Kdc1* expression in fully elongated root hairs, but did not always see expression in smaller tip growing root hairs (Fig. 2*B*), would seem to suggest that KDC1 is probably involved in the uptake of K^+ from fully elongated root hairs.

There is clearly a degree of redundancy in potassium uptake and it is highly probable that a number of K^+ channels and carriers are involved in K^+ uptake into roots, in this context it is interesting to note that a null allele of the *Arabidopsis Akt1* gene showed reduced K^+ uptake and growth only under certain growth conditions (17). Future experiments, including antisense RNA inhibition of *Kdc1,* will enable a detailed analysis of the role that this channel plays in the tip growth of root hair cells and potassium nutrition.

Acknowledgments—We thank F. Gambale and D. Pietrobon for a critical reading of the manuscript, K. Palme for access to unpublished data concerning ATKC1, M. Zoratti for the use of a patch clamp set up, and O. Pagliaccio for useful discussions.

REFERENCES

1. Clarkson, D. T., and Hanson, J. B. (1980) *Annu. Rev. Plant. Physiol.* **31,** 239–298
2. Brüggemann, L., Dietrich, P., Becker, D., Dreyer, I., Palme, K., and Hedrich, R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 3298–3302
3. Moran, N., Ehrenstein, G., Iwasa, K., Mischke, C., Bare, C., and Satter, R. L. (1988) *Plant Physiol.* **88,** 643–648
4. Schroeder, J. I., Hedrich, R., and Fernandez, J. M. (1984) *Nature* **312,** 361–362
5. Anderson, J. A., Huprikar, S. S., Kochian, L. V., Lucas, W. J., and Gaber, R. F. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 3736–3740
6. Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J. M., Gaymard, F., and Grignon, C. (1992) *Science* **256,** 663–665
7. Müller-Rober, B., Ellenberg, J., Provart, N., Willmitzer, L. Busch, H., Becker, D., Dietrich, P., Hoth, S., and Hedrich, R. (1995) *EMBO J.* **14,** 2409–2416
8. Ketchum, K. A., and Slayman, W. C. (1996) *FEBS Lett.* **378,** 19–26
9. Zimmermann, S., Talke, I., Ehrhardt, T., Nast, G., and Müller-Rober, B. (1998) *Plant Physiol.* **116,** 879–890
10. Gaymard, F., Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J., Michaux-Ferrière, N., Thibald, J., and Sentenac, H. (1998) *Cell* **94,** 647–655
11. Czempinski, K., Zimmermann, S., Ehrhardt, T., and Müller-Rober, B. (1997) *EMBO J.* **16,** 2565–75
12. Warmke, J. W., and Ganetzky, B. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 3438–3442
13. Trudeau, M., Warmke, J., Ganetzky, B., and Robertson, G. (1995) *Science* **269,** 92–95
14. Marten, I., Hoth, S., Deeken, R., Ache, P., Ketchum, K. A., Hoshi, T., and Hedrich, R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 7581–7586
15. Nakamura, R. L., Mckendree, W. L., Hirsch, R. E., Sedbrook, J. C., Gaber, R. F., and Sussman, M. R. (1995) *Plant Physiol.* **109,** 371–374
16. Lagarde, D., Basset, M., Lepetit, M., Conejero, G., Gaymard, F., Astruc, S., and Grignon, C. (1996) *Plant J.* **9,** 195–203
17. Hirsch, R. E., Lewis, B. D., Spalding, E. P., and Sussman, M. R. (1998) *Science* **280,** 918–921
18. Sambrook, J., Fritsch, E. F., and Maniatis, T., (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
19. Lipman, D. J., and Pearson, W. R. (1985) *Science* **227,** 1435–1441
20. Higgins, D. G., and Sharp, P. M. (1989) *Comput. Appl. Biosci.* **5,** 151–153
21. Logemann, J., Schell, J., and Willmitzer, L. (1987) *Anal. Biochem.* **163,** 16–20
22. Drews, G. N. (1998) in *Arabidopsis Protocols* (Martinez-Zapater, J. M., and Salinas, J., eds) Humana Press Inc., Totowa, NJ
23. Gassmann, W., and Schroeder, J. I. (1994) *Plant Physiol.* **105,** 1399–1408
24. Hamil, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) *Pflügers Arch.* **391,** 85–100
25. Neher, E. (1992) *Methods Enzymol.* **207,** 123–131
26. Engelmann, D. M., Steitz, T. A., and Goldman, A. (1986) *Annu. Rev. Biophys. Biophys. Chem.* **15,** 321–353
27. Kumpf, R. A., and Dougherty, D. A. (1993) *Science* **261,** 1708–1710
28. Glass, D. B., El-Maghrabi, M R., and Pilkis, S. J. (1986) *J. Biol. Chem.* **261,** 2987–2993
29. Woodgett, J. R., Gould, K. L., and Hunter, T. (1986) *Eur. J. Biochem.* **161,** 177–184
30. Pinna, L. A. (1990) *Biochim. Biophys. Acta* **1054,** 267–284
31. Cooper, J. A., Esch, F. S., Taylor, S. S., and Hunter, T. (1984) *J. Biol. Chem.* **259,** 7835–7841
32. Ehrhardt, T., Zimmermann, S., and Müller-Rober, B. (1997) *FEBS Lett.* **409,** 166–170
33. Daram, P., Urbach, S., Gaymard, F., Sentenac, H., and Chèrel, I. (1997) *EMBO J.* **16,** 3455–3463
34. Sedgwick, S. G., and Smerdon, S. J. (1999) *Trends Biochem. Sci.* **24,** 311–316
35. Hoth, S., Dreyer, I., Dietrich, P., Becker, D., Müller-Rober, B., and Heidrich, R. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 4806–4810
36. Hoth, S., and Hedrich, R. (1999) *J. Biol. Chem.* **274,** 11599–11603
37. Philippar, I., Fuchs I., Luthen, H., Hoth, S., Bauer C. S., Haga K., Thiel, G., Ljung, K., Sandberg, G., Bottger B., Becker, D., and Hedrich R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 12186–12191
38. Ko, C. H., and Gaber, R. F. (1991) *Mol. Cell. Biol.* **11,** 4266–4273
39. Gaymard, F., Cerutti, M., Horeau, C., Lemaillet, G., Urbach, S., Ravallec, M., Devauchellet, G., Sentenac, H., and Thibald, J. (1996) *J. Biol. Chem.* **271,** 22863–22870
40. Yu, S. P., and Kerchner, G. A. (1998) *J. Neurosci. Res.* **52,** 612–617
41. Marshall, J., Molloy, R., Moss, G. W. W., Howe, J. R., and Hughes, T. E. (1995) *Neuron* **14,** 211–215
42. Connor, J. X., Boileau, A. J., and Czakowski, C. (1998) *J. Biol. Chem.* **273,** 28906–28911
43. Lukacs, G. L., Chang, X., Bear, C., Kartner, N., Mohamed. A., Riordan, J. R., and Grinstein, S. (1993) *J. Biol. Chem.* **268,** 21592–21598
44. Felle, H., H. (1998) *J. Exp. Bot.* **49,** 987–995
45. Taylor, D. P., Slattery, J., and Leopold, A. C. (1996) *Physiol Plant.* **97,** 35–38
46. Amtmann, A., Jelitto, T. C., and Sanders, D. (1999) *Plant Physiol.* **119,** 331–338
47. Hartje, S., Zimmermann, S., Klonus, D., and Müller-Rober, B. (2000) *Planta* **210,** 723–31
48. Bibikova, T. N., Jacob, T., Dahse, I., and Gilroy, S. (1998) *Development* **125,** 2925–2934

Downloaded from http://www.jbc.org/ by guest on July 24, 2018

KDC1, a Novel Carrot Root Hair K^+Channel: CLONING, CHARACTERIZATION, AND EXPRESSION IN MAMMALIAN CELLS

Patrick Downey, Ildiko Szabò, Natalia Ivashikina, Alessandro Negro, Flavia Guzzo, Peter Ache, Rainer Hedrich, Mario Terzi and Fiorella Lo Schiavo

J. Biol. Chem. 2000, 275:39420-39426.
doi: 10.1074/jbc.M002962200 originally published online September 1, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M002962200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 26 of which can be accessed free at
http://www.jbc.org/content/275/50/39420.full.html#ref-list-1

Downloaded from http://www.jbc.org/ by guest on July 24, 2018