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Functional Expression of a Vertebrate Inwardly Rectifying K^+ Channel in Yeast

Weimin Tang, Abdul Ruknudin,* Wen-Pin Yang, Shyh-Yu Shaw, Aron Knickerbocker, and Stephen Kurtz†

Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543-4000

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We describe the expression of gpIRK1, an inwardly rectifying K^+ channel obtained from guinea pig cardiac cDNA. gpIRK1 is a homologue of the mouse IRK1 channel identified in macrophage cells. Expression of gpIRK1 in *Xenopus* oocytes produces inwardly rectifying K^+ current, similar to the cardiac inward rectifier current I_{K1}. This current is blocked by external Ba^{2+} and Cs^+. Plasmids containing the gpIRK1 coding region under the transcriptional control of constitutive (*PGK*) or inducible (*GAL*) promoters were constructed for expression in *Saccharomyces cerevisiae*. Several observations suggest that gpIRK1 forms functional ion channels when expressed in yeast. gpIRK1 complements a *trk1*Δ *trk2*Δ strain, which is defective in potassium uptake. Expression of gpIRK1 in this mutant restores growth on low potassium media. Growth dependent on gpIRK1 is inhibited by external Cs^+. The strain expressing gpIRK1 provides a versatile genetic system for studying the assembly and composition of inwardly rectifying K^+ channels.

INTRODUCTION

The transport of potassium ions is an essential function for most cells. In fungi and plants, specific transporters take up sufficient levels of potassium from the environment to accommodate intracellular ionic requirements. In animal cells, potassium-selective ion channels, in concert with the Na^+/K^+ ATPase, establish an unequal distribution of potassium across the membrane vital to the maintenance of membrane potential and for responses to excitatory signals. In *Saccharomyces cerevisiae*, potassium uptake is accomplished by specific transporters whose function ensures uptake in growth conditions where potassium is in low abundance (Rodriguez-Navarro and Ramos, 1984; Ramos *et al*, 1985). An electrochemical gradient established by the plasma membrane H^+ ATPase provides energy for K^+ uptake and potassium is taken up as a counterion for H^+ (Serrano, 1991). Gaber and coworkers have defined the molecular components mediating K^+ uptake in yeast. Two loci, *TRK1* and *TRK2*, encode high and low affinity K^+ transporters, respectively (Gaber *et al.*, 1988; Ko *et al.*, 1990; Gaber, 1992). Strains containing mutations in both transporters are severely impaired for K^+ uptake and require media supplemented with KCl for growth (Ko and Gaber, 1991). Complementation of potassium uptake mutants with cDNAs encoding functions that restore growth on potassium-deficient media have identified two distinct K^+ channels from *Arabidopsis thaliana* (Anderson *et al.*, 1992; Sentenac *et al.*, 1992) and a K^+ transporter from *Triticum aestivum* (Schachtman and Schroeder, 1994).

The K^+ channel superfamily comprises a diverse set of ion-selective membrane proteins. Since the elucidation of Shaker, the structure of a voltage-gated potassium channel has emerged as a functional molecule consisting of four subunits, each of which contains six potential membrane-spanning domains (Papazain *et al.*, 1987; Kamb *et al.*, 1987). A pore region essential for potassium permeation is the primary feature conserved among all K^+ channels (Jan and Jan, 1992; Brown, 1993). Subsequent advances in molecular cloning have defined the inward rectifiers, a subfamily of K^+ channels distantly related to the voltage-gated K^+ channels (Dascal *et al.*, 1993; Ho *et al.*, 1993; Kubo *et al.*, 1993a,b; Bond *et al.*, 1994; Tang and Yang, 1994). Sequence analysis indicates that these channels contain

* Present address: Department of Microbiology & Immunology, University of Maryland Medical School, 655 W. Baltimore St., Baltimore, MD 21201.
† Corresponding author.

two, rather than six, potential transmembrane domains, and with the exception of the potassium-selective pore region, there is little primary sequence identity between inwardly rectifying K^+ channels and voltage-gated K^+ channels.

Inwardly rectifying K^+ channels are functionally distinct from voltage-gated K^+ channels in that their conductance is increased by hyperpolarization rather than by membrane depolarization (Hille, 1992; Jan and Jan, 1994). Voltage-gated channels are activated at membrane potentials positive to the resting potential and pass outward K^+ current (Iverson *et al.*, 1988; Timpe *et al.*, 1988). In contrast, inwardly rectifying K^+ channels open with hyperpolarizing voltages and permit inward K^+ flux at membrane potentials below the potassium equilibration potential (E_K). Inward rectifiers are closed upon depolarization and consequently function in ventricular myocytes to prolong action potentials and in oocytes to prevent double fertilization (Hagiwara and Jaffe, 1979; Hille, 1992; Kass and Freeman, 1993). However, some inwardly rectifying K^+ channels also pass minimal outward current at membrane potentials just above E_K, which serves to maintain resting potential (Hille, 1992).

The observations that plant K^+ channels function in yeast suggests that a similar strategy may be applicable to vertebrate K^+ channels. Because guinea pig cardiac myocytes are widely used to study various ionic currents and to test drugs that affect ion channels, we cloned a member of the inward rectifier K^+ channel family from guinea pig cardiac cDNA. Voltage clamp analysis of gpIRK1 cRNA expressed in *Xenopus* oocytes indicates current with properties typical of the inward rectifier observed in guinea pig cardiac tissue (Kurachi, 1985). We expressed gpIRK1 in *S. cerevisiae* and observed that its ion channel function complements a mutant defective in potassium uptake. These data indicate that the inwardly rectifying class of vertebrate K^+ channels can substitute functionally for fungal potassium transporters.

MATERIALS AND METHODS

Cloning of a Guinea Pig Inward Rectifier K^+ Channel

A DNA fragment of 1.3 kb was amplified in a polymerase chain reaction (PCR) using guinea pig heart cDNA with primers homologous to the mouse IRK1 coding region: 5′-CGCGGATCCCG-GAAGCCAATGGGCAGT-3′ and 5′-CGCGGAT-CCAGCCAGT-CATATCTCCGA-3′. The fragment was radiolabeled and used to identify hybridizing clones from a guinea pig genomic library (Stratagene, La Jolla, CA). Approximately 500,000 phage plaques containing a guinea pig genomic library were screened and single plaques were obtained after two subsequent rounds of hybridization. DNA sequence analysis was performed on three independent isolates using an ABI Sequenator. The EMBL accession number for gpIRK1 is Z48971.

Northern blot hybridizations were performed on poly·(A+) mRNAs isolated from guinea pig heart tissue. RNAs were separated by electrophoresis through a 1% agarose-formaldehyde gel, transferred to NYTRAN membrane (Schleicher & Schuell, Keene, NH), and hybridized with a probe specific for the gpIRK1 coding region that had been radiolabeled by random priming in the presence of [α-^{32}P]dCTP (3000 Ci/mmol; NEN, Boston, MA). The blot was exposed for 3 days at −80°C. RNA markers were visualized by trimming a lane from the blot and staining with methylene blue.

Electrophysiological Measurements

Plasmid DNA containing gpIRK1 was subcloned into the PCRII vector, linearized, and transcribed in vitro with SP6 RNA polymerase using the mMESSAGE MACHINE (Ambion, Austin, TX). gpIRK1 cRNA was extracted and resuspended at a concentration of 0.4 mg/ml for injection into *Xenopus* oocytes.

Oocytes were impaled with standard borosilicate glass microelectrodes with resistances ranging from 0.5–1.5 MΩ when filled with 3 M KCl. Whole cell currents were recorded from oocytes using a two-electrode voltage clamp with a Turbo TEC-01C amplifier (NPI Instruments, Tamm, Germany) and Axodata software; data analyses were performed with Axograph software. Recordings were performed at 22–24°C 2 days after RNA injection. Oocytes were superfused continuously in ND-96 containing (in mM): NaCl, 96; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 5; pH to 7.4 with NaOH. In solutions with different K^+ concentrations, the KCl concentration was varied with proportionate adjustments in NaCl concentrations. $BaCl_2$ and CsCl, dissolved in ND-96, were applied externally by changing the bath solution. Oocytes were held at −45 mV and depolarization pulses were applied for 300 ms with a 1 s interval between pulses. Steady state current was measured 282 ms after the beginning of the pulse. All currents measured were steady-state except where indicated. Transient currents were measured 12 ms after the initiation of the pulse. Leak currents were subtracted by pulsing to depolarizing potentials and then subtracting a linearly scaled inverted pulse from the inward current. Currents are representative of recordings from a minimum of six oocytes, except in Figure 3B in which recordings are representative of four oocytes.

Yeast Strains and Manipulations

Strains were constructed in both mating types of W303 as follows: *ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1*. Expression plasmids were introduced into strain SGY1528 as follows: *MATa ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1 trk1::HIS3 trk2::TRP1*.

Strains used in this study were as follows:
SGY1529: contains parent vector A241 (2 μ-*URA3-pPGK*);
SGY1530: contains A241 with *PGK::gpIRK1*;
SGY1531: contains A241 with *PGK::TRK1*;
SGY1532: contains parent vector pYES2 (2 μ-*URA3-pGAL1*);
SGY1533: contains pYES2 with *GAL::gpIRK1*.

Construction of K^+ Transporter Mutants

The 5′ and 3′ ends of the *TRK1* coding region were amplified in a PCR using yeast genomic DNA and two sets of oligonucleotides specific to *TRK1*. These fragments were radiolabeled and used to identify hybridizing clones from a yeast genomic library.
Set #1: 5′ GCATTTTAGAAGAACGATGAGTAGAGTGCCC 3′
5′ AGACGTTCTTTGGGTACCTGTTCTATTCTTGG 3′
Set #2: 5′ GACGAGGAAAACGAGAGTCACGAAGG 3′
5′ GAGCGTTGTGCTGCTCCTTTTAGGATTTCG 3′

Sequences containing the entire *TRK1* locus were subcloned into pUC19 and a deletion removing 2350 bp of the *TRK1* coding region was created by digestion with *Xba*I. A nonfunctional allele *trk1::HIS3* was constructed by insertion of the *HIS3* gene into the *Xba*I site and used to disrupt the *TRK1* locus using the one-step method (Rothstein, 1983). The disruption was verified by Southern

blot analysis. Transformants containing the *trk1::HIS3* allele require media supplemented with potassium for normal growth.

Portions of the *TRK2* locus corresponding to the 5′ (600 bp) and 3′ (800 bp) segments of the coding region were amplified in a PCR using yeast genomic DNA and two sets of oligonucleotides specific to *TRK2*. The fragments were assembled in a three-component ligation with pSL1180 (Pharmacia, Piscataway, NJ).

Set #1: 5′ GCCAACAGCTAAGAGGACGTCATCC 3′
5′ CTTCGAGCGGCATTGCCACTGCTGCC 3′
Set #2: 5′ GTCCAAGCCGAAGAAACAGTCCCC 3′
5′ GCTTCCCCCAAAACTTTGTTGC 3′

The resulting plasmid was linearized at the junction of the 5′ and 3′ *TRK2* coding fragments and religated with a fragment containing the yeast *TRP1* gene. This construction, *trk2::TRP1*, lacks 1170 bp of the *TRK2* coding region and was used to disrupt the *TRK2* locus. The disruption was verified by Southern blot analysis. Transformants containing the *trk2::TRP1* allele do not have a growth phenotype on low K^+ media.

The double mutant *trk1Δtrk2Δ* was obtained by isolating His+Trp+ segregants from a *MATa trk1::HIS3* × *MATα trk2::TRP1* cross. As expected, the K^+ uptake defect is more severe in segregants containing both mutations than in those with either single mutation.

Construction of Expression Plasmids

The gpIRK1 coding region was inserted under the transcriptional control of the constitutive promoter for phosphoglycerate kinase (*PGK*) to construct *PGK::gpIRK1* in plasmid A241. Plasmid A241 also contains a 2μ origin of replication and the *URA3* gene. A similar construct was made with the *GAL1* promoter to form *GAL::gpIRK1* in pYES2 (Invitrogen, San Diego, CA). The following oligonucleotides were used to amplify sequences encoding gpIRK1 from a full length cDNA clone: 5′-TAGGATCCGAAAAAAAATGGGCAGTGTGCGAACCAAC-3′ and 5′-TAGAATTCAGTCATATCTCCGATTCTCGCCG-3′. The PCR-amplified product was digested with *Bam*HI and *Eco*RI and cloned into identically digested A241 or pYES2 vectors. Plasmids were verified by restriction digests and by DNA sequencing.

The *TRK1* coding region was amplified in a PCR using 5′ CGGGATCCAAAAAATGCATTTTAGAAGAACGATGAG 3′ and 5′ CCCGGCATGCCGATGAGTGGGGATTTTGTC 3′, digested with *Bam*HI and *Sph*I, and placed under the transcriptional control of the *PGK* promoter to construct *PGK::TRK1*, in identically digested A241. Plasmids were verified by restriction digests and by DNA sequencing. The plasmid containing *PGK::TRK1* complements the K^+ uptake defect of the *trk1::HIS3* mutant.

Plasmids were introduced into cells made competent for transformation using the lithium acetate protocol (Ito *et al.*, 1983). Transformants were maintained on selective media containing 2% glucose (noninducing conditions) and 100 mM KCl.

Growth Assays

Standard yeast media were used for cell growth (Sherman *et al.*, 1983). Powdered minimal media stocks were obtained from BIO 101 (La Jolla, CA). Minimal media containing 2% galactose was used to induce expression of *GAL::gpIRK1*. Strains containing expression plasmids were grown overnight in minimal media containing 2% glucose and 100 mM KCl. For growth assays, cells were washed once in sterile water, resuspended at 10^5 cells/ml in low potassium media, and grown at 30°C with vigorous aeration. Optical density was determined at intervals by measuring absorbance at 600 nm. Inhibitors were added to cultures at the time of induction over a range of concentrations. For plate assays, equivalent numbers of cells from overnight cultures were spotted onto plates and incubated at 30°C for 2–3 days.

RESULTS

Cloning of an Inward Rectifying K^+ Channel from Guinea Pig Heart

Oligonucleotide primers based on the sequence of the mouse IRK1 gene (Kubo *et al.*, 1993a) were used in a PCR to amplify a 1.3-kb DNA product from guinea pig heart cDNA. This product was then used to identify homologous sequences by hybridization to λ phage plaques containing a guinea pig genomic library. Sequence analysis of the hybridizing clones indicated an open reading frame of 1281 nucleotides corresponding to gpIRK1.

The nucleotide sequence of gpIRK1 encodes a predicted polypeptide of 427 amino acids (Figure 1). The gene encoding gpIRK1 is intronless as PCR amplification from a genomic DNA template produced a product identical in size to the coding region present on the full length cDNA. At the nucleotide level, this sequence is 87% identical to the mouse IRK1 nucleotide sequence. Sequence features in IRK1 are also present in gpIRK1: a pore region with homology to the pore region of K^+ selective ion channels (H5) lies between two putative transmembrane domains (M1 and M2). Although gpIRK1 is one amino acid smaller than IRK1 (it lacks a glutamic acid residue at position 389), the predicted amino acid sequence of the gpIRK1 protein is nearly identical (99%) to that for IRK1. Six amino acid differences occur between the predicted mouse and guinea pig proteins and these changes are contained primarily within the C-terminus. Hybridization with a radiolabeled probe derived from the gpIRK1 coding region revealed a single hybridizing species of approximately 5.5 kb in poly(A+) mRNAs isolated from total guinea pig cardiac tissue (Figure 2).

Expression of gpIRK1 in Xenopus Oocytes

Current obtained from two-electrode voltage clamp recordings of *Xenopus* oocytes injected with cRNAs for gpIRK1 exhibits properties characteristic of inwardly rectifying K^+ channels. The expressed current was activated by potentials below E_K (Figure 3A). At potentials positive to E_K, only minimal outward current was produced. No significant inward current was recorded from water-injected oocytes under similar conditions (our unpublished observations). gpIRK1 current exhibits a nonlinear dependence in conductance on extracellular K^+ concentration $[K+]_e$, typical of that seen in other inward rectifiers (Kubo *et al.*, 1993a). The slope conductance varies with increased $[K+]_e$. The reversal potential changed 57.5 mV with a 10-fold change in $[K+]_e$, indicating that the *gpIRK1* channel is highly selective for potassium (Figure 3B).

The current-voltage relationships for gpIRK1 shows large inward current at negative potentials relative to E_K. In bath solutions containing 2 mM K^+, there is a

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gpIRK1 MGSVRTNRYSIVSSEEDGMKLATMAVANGFGNGKSKVHTRQQCRSRFVKK 50
  IRK1 :::::::::::::::::::::::::::::::::::::::::::::::::: 50
                                                  M1
gpIRK1 DGHCNVQFINVGEKGQRYLADIFTTCVDIRWRWMLVIFCLAFVLSWLFFG 100
  IRK1 :::::::::::::::::::::::::::::::::::::::::::::::::: 100
                                              H5
gpIRK1 CVFWLIALLHGDLDASKESKACVSEVNSFTAAFLFSIETQTTIGYGFRCV 150
  IRK1 ::::::::::::::T::V:::::::::::::::::::::::::::::::: 150
                  M2
gpIRK1 TDECPIAVFMVVFQSIVGCIIDAFIIGAVMAKMAKPKKRNETLVFSHNAV 200
  IRK1 :::::::::::::::::::::::::::::::::::::::::::::::::: 200

gpIRK1 IAMRDGKLCLMWRVGNLRKSHLVEAHVRAQLLKSRITSEGEYIPLDQIDI 250
  IRK1 :::::::::::::::::::::::::::::::::::::::::::::::::: 250

gpIRK1 NVGFDSGIDRIFLVSPITIVHEIDEDSPLYDLSKQDIDNADFEIVVILEG 300
  IRK1 :::::::::::::::::::::::::::::::::::::::::::::::::: 300

gpIRK1 MVEATAMTTQCRSSYLANEILWGHRYEPVLFEEKHYYKVDYSRFHKTYEV 350
  IRK1 :::::::::::::::::::::::::::::::::::::::::::::::::: 350

gpIRK1 PNTPLCSARDLAEKKYILSNANSFCYENEVALTSK.EEDDSENGVPESTS 399
  IRK1 ::::::::::::::::::::::::::::::::::E::E:::::::::::: 400

gpIRK1 TDTPPDIDLHNQASVPLEPRPLRRESEI 427
  IRK1 ::S::G:::::::::::::::::::::: 428
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Figure 1. Predicted amino acid sequence of gpIRK1 aligned with mouse IRK1. Lines denote the transmembrane regions (denoted M1 and M2) and the pore (denoted H5). Dots represent identical amino acid residues.

pronounced reduction in steady-state current (Figure 4A). The reduction of inward current observed in the 2 mM K^+ solution may result from external Na^+, which is 96 mM, and is reminiscent of the Na^+ block observed on inwardly rectifying K^+ current in guinea pig myocytes (Biermans *et al.*, 1987). Current produced in oocytes injected with gpIRK1 cRNAs was blocked by external Ba^{2+} and Cs^+ (10 and 100 μM) in the presence of 2 mM K^+ (Figure 4, B and C). Ba^{2+} was a more efficient blocker at 10 μM, although both cations block the channels almost completely at 100 μM. The relative effectiveness of blocking gpIRK1 by external cations is $Ba^{2+} > Cs^+ > Na^+$. The block by external cations manifests as inactivation in a time- and voltage-dependent manner similar to previous observations in guinea pig cardiac myocytes (Mitra and Morad, 1991).

gpIRK1 Complements a Yeast Strain Defective in Potassium Uptake

Potassium uptake in *S. cerevisiae* is mediated by transporter proteins with specific affinity for K^+. Two genes, *TRK1* and *TRK2*, encode high and low affinity transporters, respectively (Gaber *et al.*, 1988; Ko and Gaber, 1991). Strains containing loss-of-function mutations in both genes are unable to grow on standard yeast media and require media supplemented with millimolar levels of potassium (100 mM) for normal growth. Transporter-defective strains have been used to isolate genes encoding activities from *A. thaliana* that rescue the growth defect on potassium-deficient media (Anderson *et al.*, 1992; Sentenac *et al.*, 1992; Schachtman and Schroeder, 1994). Because inward rectifying K^+ channels allow K^+ influx in hyperpolarized membranes (a condition satisfied by the yeast cell) (Bakker *et al.*, 1986; Pena *et al.*, 1987), we reasoned that gpIRK1 would complement a K^+ uptake mutant and restore growth on low potassium media.

We inserted the gpIRK1 coding region under the transcriptional control of two yeast promoters and introduced plasmids containing these constructions into a K^+ transporter mutant strain. High copy expression plasmids were constructed with both constitutive (*PGK*) and inducible (*GAL*) yeast promoters. Plasmids were transformed into strain SGY 1528, which is defective in K^+ uptake because the coding region at each transporter locus had been deleted. Sixteen independent transformants for each construction were assayed for growth on potassium-deficient media. For both promoter constructions, all 16 transformants grew on potassium-deficient media, indicating complementation of the uptake defect. Transformants expressing gpIRK1 from the *PGK* promoter grew on media containing low levels of potassium (Figure 5A). A control strain containing the vector did not grow on potassium-deficient media and a strain expressing the yeast

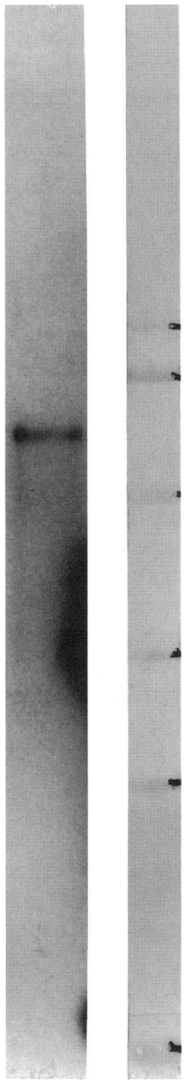


Figure 2. Detection of gpIRK1 mRNAs. Guinea pig poly-(A+) heart mRNAs were separated by electrophoresis through a 1% agarose-formaldehye gel, transferred to a NYTRAN membrane, and hybridized with a 1.3-kb radiolabeled probe specific for the gpIRK1 coding region. RNA size markers are as follows: 9.5, 7.5, 4.4, 2.4, 1.4, and 0.24 kb (right lane).

Trk1 K^+ transporter from the *PGK* promoter grew on both media, as expected.

The growth of strains expressing gpIRK1 on low potassium media was not as vigorous as that observed in a control strain expressing the yeast K^+ transporter *TRK1*. Growth measurements indicate that the gpIRK1-expressing strain has reduced growth relative to the *TRK1* expressing control (Figure 5B). A strain containing the parental vector did not grow in these conditions. Determination of growth rates for the strain expressing the gpIRK1 indicates that it restores growth rates to approximately 50% of the level observed for the strain expressing the yeast *TRK1* transporter from a similar plasmid. The doubling time of the gpIRK1-expressing strain is 4 h compared with 2 h for the *TRK1*-expressing strain. Thus, the expression of gpIRK1 provides a partial rescue of the potassium uptake defect.

To confirm that the function complementing the defect in K^+ uptake was plasmid dependent, strains containing the gpIRK1 expression plasmids were grown nonselectively in potassium-rich medium to facilitate plasmid loss. Colonies having lost the plasmid were identified by growth on media containing 5-fluoro-orotic acid and then assessed for growth in potassium-deficient medium. In the absence of the gpIRK1 expression plasmid, cells did not grow on potassium-deficient media, indicating that the growth observed on low potassium media was dependent on a plasmid-encoded function. Retransformation of the plasmid-minus strain with the initial gpIRK1 expression plasmid restored growth on low potassium medium, indicating complementation of the defect in potassium uptake (our unpublished observations).

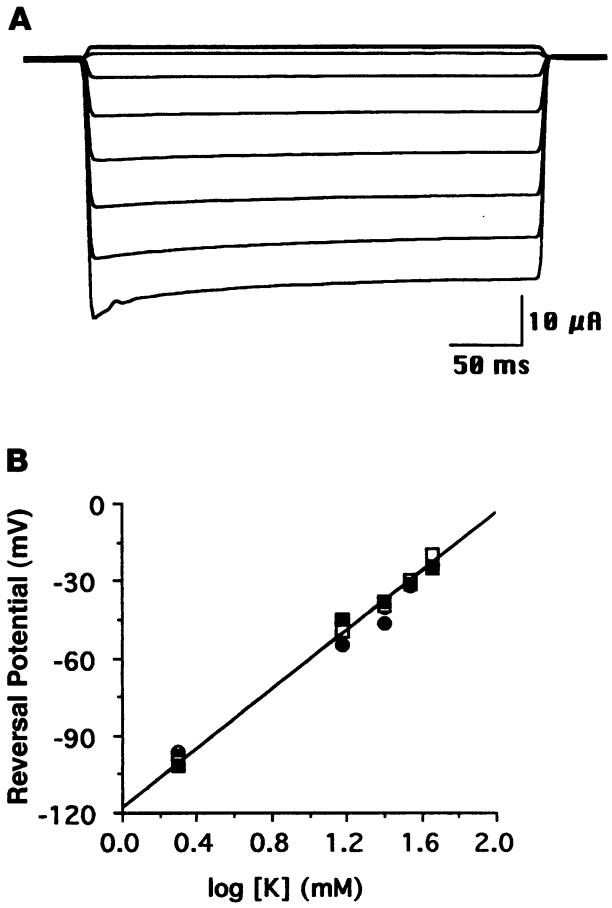
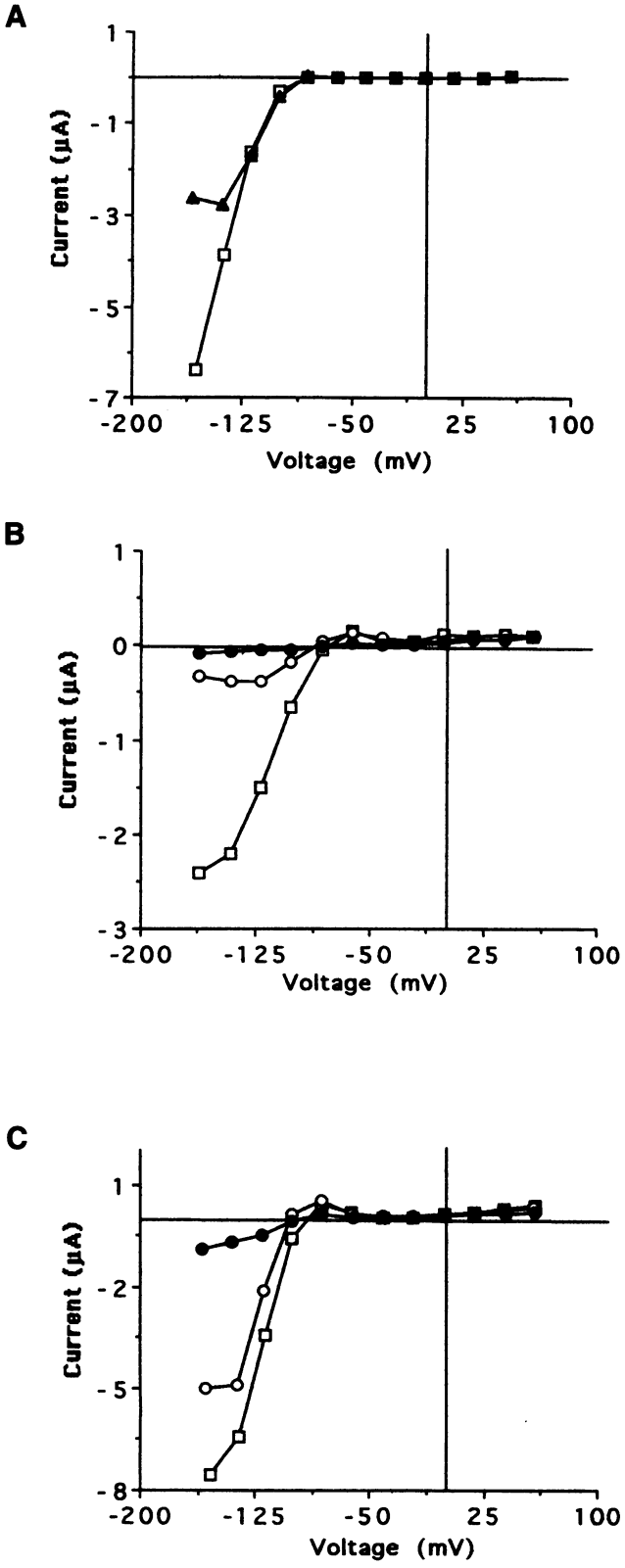


Figure 3. (A) Expression of gpIRK1 in *Xenopus* oocytes. Current traces recorded by two-electrode voltage clamping of oocytes injected with gpIRK1 mRNAs. Oocytes were held at a potential of −45 mV and current was elicited by applying 300-ms pulses ranging from −160 to 0 mV in 20-mV intervals. The bath solution contained 34 mM KCl in ND-96. (B) The reversal potential of gpIRK1 current plotted as a function of external K^+ concentration obtained from steady-state current measurements (n = 4). The straight line is the best fit of the data to the Nernst equation with a slope of 57.49 mV.



Transformants expressing *GAL::gpIRK1* grew on media containing low levels of potassium in the presence of galactose, but not on similar plates containing glucose, indicating that expression of gpIRK1 from an inducible promoter also provides restoration of growth on low potassium media (our unpublished observations). A control strain containing the vector did not grow on potassium-deficient media regardless of the carbon source.

External Cesium Blocks gpIRK1 Function in Yeast

The strain expressing gpIRK1 was grown in the presence of external cations to assess their effects on growth. External Ba^{2+} and Cs^+ block many potassium selective channels, including the inward rectifier K^+ channel (Kubo *et al.*, 1993a; Bond *et al.*, 1994; this study). On agar plates containing 1 mM CsCl, strains expressing gpIRK1 were completely inhibited for growth (Figure 6A). Growth of the *TRK1*-expressing strain was unaffected by the presence of Cs^+ ions. Inclusion of potassium (100 mM) in media containing Cs^+ ions supported growth for strains containing either the vector, gpIRK1, or *TRK1* plasmids, indicating that cesium does not by itself impair yeast growth. The results with barium were inconclusive as $BaCl_2$ is insoluble in standard yeast media. Growth of the strain expressing gpIRK1 from the *GAL* promoter was similarly inhibited by Cs^+.

To evaluate the effect of Cs^+ on the gpIRK1-expressing strain, cells were grown in media containing high levels of potassium and then shifted to low potassium media in the presence of varying concentrations of CsCl. Cs^+ inhibits the growth of the strain expressing gpIRK1 in a selective and concentration-dependent manner; complete inhibition was observed at concentrations of 500 μM (Figure 6B). Similar assays were performed in the presence of varying concentrations of Mg^{2+}, Na^+, Rb^+, and TEA up to 100 mM. Neither Mg^{2+}, Na^+, nor Rb^+ were inhibitory to the growth of the gpIRK1-expressing strain. TEA produced a modest inhibition at concentrations above 10 mM (our unpublished observations). None of these ions had an effect on the growth of a control strain that expresses the yeast transporter *TRK1* from a similar plasmid, nor did these ions affect the growth of the gpIRK1-expressing strain in the presence of 100 mM KCl. Addition of increasing concentrations of K^+ in the presence of Cs^+ shifted the growth inhibition in the gpIRK1-expressing strain in a competitive manner, such that higher

Figure 4. Current-voltage relationships indicating the voltage-dependent block of gpIRK1 in the presence of external Na^+ (A), Ba^{2+} (B), or Cs^+ (C). The bath solution contained 2 mM KCl. In panel A, □ denotes transient currents, and ▲ denotes steady-state currents. In panels B and C, $BaCl_2$ and CsCl concentrations were 0 (□), 10 (○), and 100 μM (●).

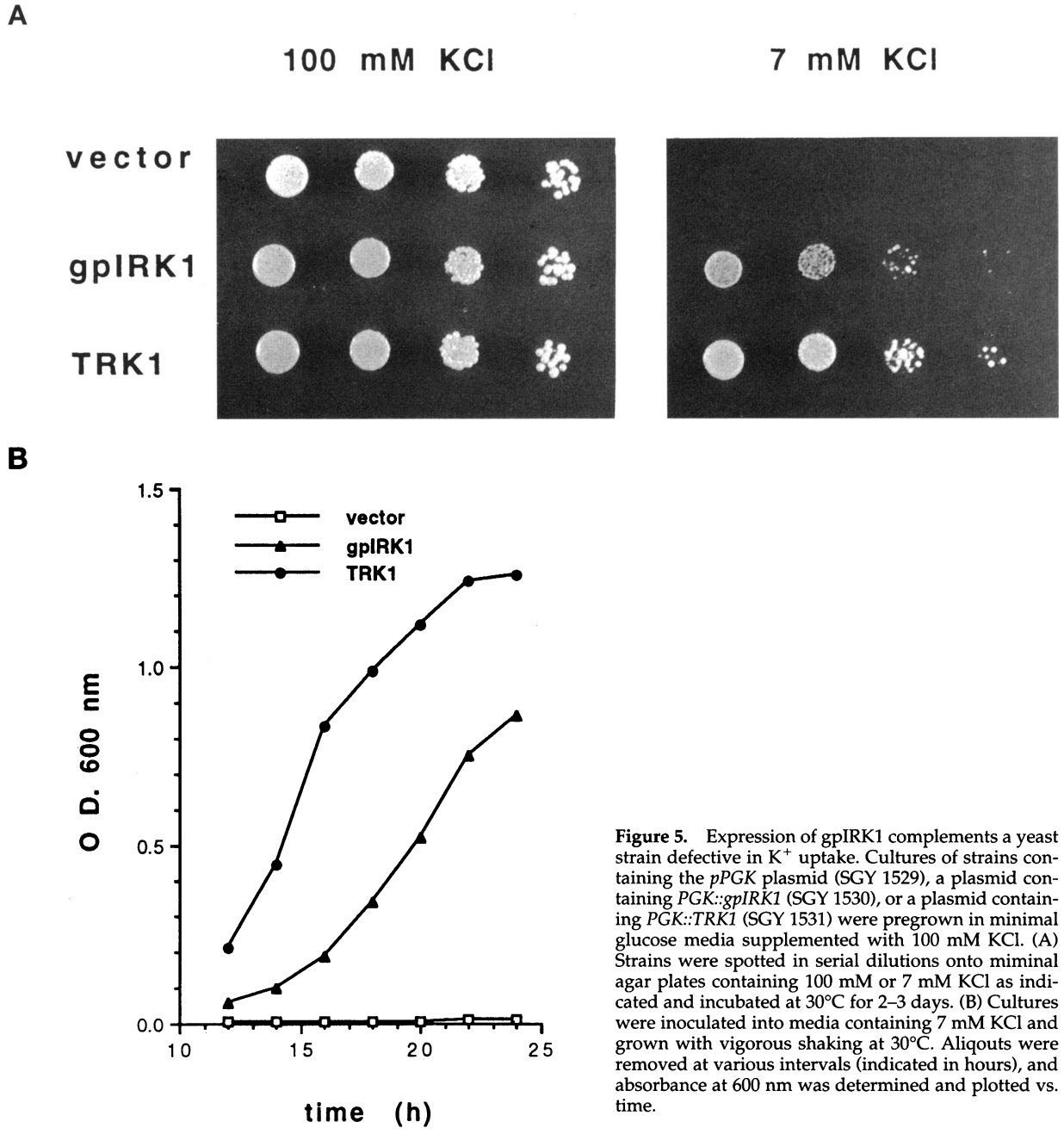


Figure 5. Expression of gpIRK1 complements a yeast strain defective in K^+ uptake. Cultures of strains containing the *pPGK* plasmid (SGY 1529), a plasmid containing *PGK::gpIRK1* (SGY 1530), or a plasmid containing *PGK::TRK1* (SGY 1531) were pregrown in minimal glucose media supplemented with 100 mM KCl. (A) Strains were spotted in serial dilutions onto miminal agar plates containing 100 mM or 7 mM KCl as indicated and incubated at 30°C for 2–3 days. (B) Cultures were inoculated into media containing 7 mM KCl and grown with vigorous shaking at 30°C. Aliqouts were removed at various intervals (indicated in hours), and absorbance at 600 nm was determined and plotted vs. time.

levels of cesium were required to inhibit the strain growing in conditions with increased levels of potassium (Figure 6C).

DISCUSSION

To establish a system for studying K^+ channel function that expands on the expression of plant K^+ channels in yeast, we isolated a member of the inward rectifier K^+ channel family from guinea pig cardiac cDNA. Expression of this clone in *Xenopus* oocytes produces inwardly rectifying K^+ current typical of the inward rectifier observed in guinea pig cardiac tissue (Kurachi, 1985). Consistent with other known inward rectifier K^+ channels, gpIRK1 current is induced by voltages negative to E_K. The reversal potential of gpIRK1 current changes with the extracellular K^+ concentration, indicating that

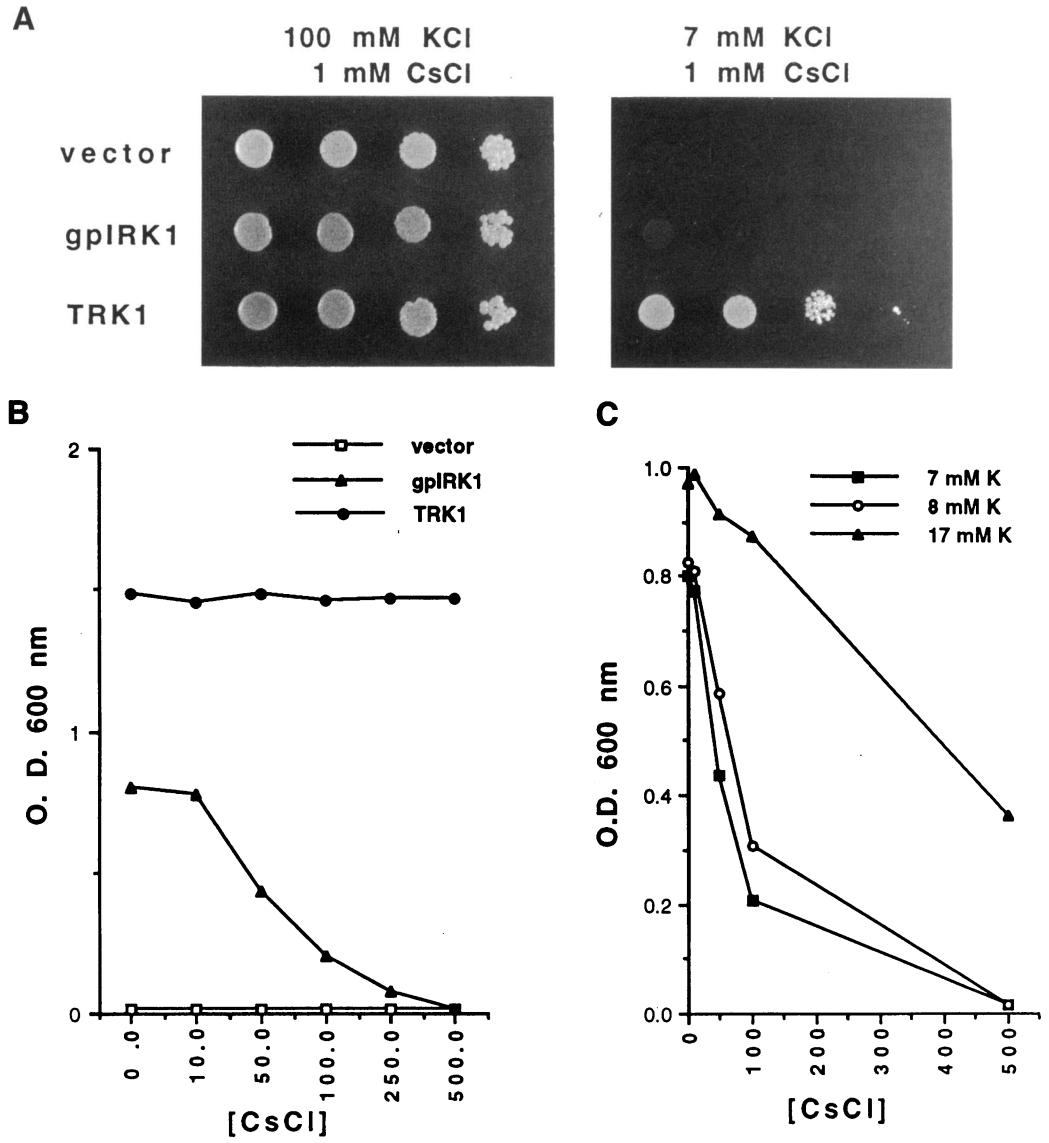


Figure 6. Cesium blocks K^+ uptake in yeast cells expressing gpIRK1. Cultures of strains containing the *pPGK* plasmid (SGY 1529), *pPGK::gpIRK1* (SGY 1530), or *pPGK::TRK1* (SGY 1531) were pregrown in minimal media lacking uracil and supplemented with 100 mM KCl. (A) Strains were spotted in serial dilutions onto miminal agar plates containing 1 mM CsCl and either 100 mM or 7 mM KCl, as indicated. Plates were incubated at 30°C for 2–3 days. (B) Strains were inoculated into media containing 7 mM KCl and increasing concentrations of CsCl (10, 50, 100, 250, and 500 μM) and grown with vigorous shaking at 30°C for 20 h. Absorbance at 600 nm was determined and plotted vs. Cs^+ concentration. (C) Strain SGY 1530 containing *pPGK::gpIRK1* was inoculated into media containing 7, 8, or 17 mM KCl with increasing concentrations of CsCl (10, 50, 100, and 500 μM) and grown with vigorous shaking at 30°C for 20 h. Absorbance at 600 nm was determined and plotted vs. Cs^+ concentration.

this channel is potassium selective. Voltage- and time-dependent block of gpIRK1 with external Ba^{2+} and Cs^+ indicates that these cations act as open channel pore blockers. This is consistent with blockade of the guinea pig cardiac inwardly rectifying current I_{K1}, with Ba^{2+} and Cs^+ (Imito *et al.*, 1987; Mitra and Morad, 1991). The reduction in gpIRK1 current observed with external Na^+ is more pronounced at hyperpolarizing voltages, reminiscent of previous observations on guinea pig ventricular myocytes (Biermans *et al.*, 1987).

The expression of gpIRK1 in yeast complements a defect in K^+ uptake characteristic of *trk1Δtrk2Δ* cells. The growth restoration is plasmid-dependent as loss of the gpIRK1-containing plasmid results in loss of growth on potassium-deficient medium. Furthermore, the expression of gpIRK1 in yeast is promoter independent; both constitutive and inducible promoters provide sufficient expression of gpIRK1 ion channels to rescue growth. The rescue observed with gpIRK1 expression is partial with respect to the rate of potassium uptake generated by the yeast K^+ transporter *Trk1*. In plate assays, growth of the strain rescued by expression of gpIRK1 is reduced relative to that observed for the strain expressing *TRK1* after 2 days of incubation. Determination of growth rates similarly reflects this partial rescue. The partial growth rescue may reflect variations in expression levels as well as inherent functional differences between gpIRK1 and *Trk1*. We also cannot eliminate the possibility that gpIRK1 subunits associate with an endogenous yeast protein in a manner that interferes with complete

growth rescue. However, we have no evidence that IRK1-like proteins exist in yeast, and patch clamp analysis indicates that *trk1Δ trk2Δ* cells are devoid of inward current (Bertl *et al.*, 1995), an observation that suggests that yeast does not contain proteins, except for *Trk1* and *Trk2*, with inward rectification properties.

The complementation data suggest that the restoration of potassium uptake in yeast cells expressing gpIRK1 results from the formation of functional inwardly rectifying K^+ channels. Further evidence supporting the formation of gpIRK1 channels is derived from inhibition studies with external Cs^+, which blocks growth of the gpIRK1-expressing strain in a selective and concentration-dependent manner. Additionally, there is a direct correlation between the amount of Cs^+ required to inhibit growth of the gpIRK1-expressing strain and the potassium concentration. It is likely that gpIRK1 rescues growth on potassium-limiting medium because the channel inserts in a hyperpolarized membrane and consequently permits an inward K^+ flux. Due to the proton-extruding activity of the plasma membrane H^+ ATPase, the yeast cell membrane is polarized to approximately −180 mV (Bakker *et al.*, 1986; Pena *et al.*, 1987) and satisfies a requirement for activation of IRK1-type K^+ channels (Ho *et al.*, 1993; Kubo *et al.*, 1993a). A direct demonstration of channel formation will require patch clamp analysis of yeast membranes prepared from the gpIRK1-expressing strain.

The growth rescue obtained by expression of gpIRK1 in the transporter-defective yeast strain is similar to that observed for expression of cDNAs encoding the *A. thaliana* K^+ channels *KAT1* and *AKT1* (Anderson *et al.*, 1992; Sentenac *et al.*, 1992). Electrophysiological analysis of *KAT1* expression in *Xenopus* ooctyes revealed inwardly rectifying K^+ currents that are activated by hyperpolarization (Schachtman *et al.*, 1992). In light of the inward rectification properties demonstrated for *KAT1*, it is not surprising that functionally analogous IRK1-type K^+ channels restore K^+ uptake in yeast. Curiously, the *AKT1* and *KAT1* cDNAs encode proteins that have similarity with outward rectifying K^+ channels of the Shaker family in regions thought to be responsible for voltage sensing and ion selectivity.

The strain expressing gpIRK1 has several advantages for studying ion channel functions. Because this strain is genetically stable, it is amenable to development into a high capacity screen for identifying compounds that modulate gpIRK1 channel function. The utility of the strain as a screening method is validated by the inhibition of cell growth observed in the presence of Cs^+. Expression of gpIRK1 in yeast opens the possibility of applying genetic methods to study inward rectifier function. Residues essential for gpIRK1 function may be defined by mutagenesis of the plasmid containing gpIRK1 and identified by alterations in growth on low potassium media or Cs^+ sensitivity (Sikorski and Boeke, 1991). This strain may also provide a means of studying multimer assembly by coexpression of components that associate to form channels. The importance of this feature is underscored by recent experiments demonstrating the heteromultimeric nature of an atrial inwardly rectifying K^+ channel (Krapivinsky *et al.*, 1995). From studies using *Xenopus* oocytes, the atrial current I_{KACh} is obtained by coexpression of two related polypeptides, GIRK1 and CIR, whereas expression of either subunit alone produced homomultimeric channels with substantially smaller current. It may be possible to detect heteromultimers by coexpressing known channel-encoding genes or expression libraries.

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