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# Molecular Characterization of an Inwardly Rectifying $K^+$ Channel from HeLa Cells

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**Abstract.** Previous patch-clamp studies have shown that the potassium permeability of the plasma membrane in HeLa cells, a cell line derived from an epidermoid carcinoma of the cervix, is controlled by various $K^+$-selective pores including an IRK1 type inwardly rectifying $K^+$ channel. We used the sequence previously reported for the human heart Kir2.1 channel to design a RT-PCR strategy for cloning the IRK1 channel in HeLa cells. A full-length clone of 1.3 kb was obtained that was identical to the human cardiac Kir2.1 inward rectifier. The nature of the cloned channel was also confirmed in a Northern blot analysis where a signal of 5.3 kb corresponding to the molecular weight expected for a Kir2.1 channel transcript was identified not only in HeLa cells, but also in WI-38, ECV304 and bovine aortic endothelial cells. The HeLa IRK1 channel cDNA was subcloned in an expression vector (pMT21) and injected into *Xenopus* oocytes. Cell-attached and inside-out single channel recordings obtained from injected oocytes provided evidence for a voltage-independent $K^+$-selective channel with current/voltage characteristics typical of a strong inward rectifier. The single channel conductance for inward currents measured in 200 mM $K_2SO_4$ conditions was estimated at 40 ± 1 pS ($n = 3$), for applied voltages ranging from −100 to −160 mV, in agreement with the unitary conductance for the IRK1 channel identified in HeLa cells. In addition, the single channel conductance for inward currents, $\Gamma$, was found to vary as a function of $\alpha_K$, the external $K^+$ ion activity, according to $\Gamma = \Gamma_0 [\alpha_K]^\delta$ with $\Gamma_0 = 3.3$ pS and $\delta = 0.5$. Single channel recordings from injected oocytes also provided evidence of a voltage-dependent block by external $Cs^+$ and $Ba^{2+}$. The presence of 500 μM $Cs^+$ caused a voltage-dependent flickering, typical of a fast channel blocking process which resulted in a reduction of the channel open probability at increasingly negative membrane potential values. The fractional electrical distance computed for the $Cs^+$ blocking site was greater than 1 indicating a multiple ion channel occupation. In contrast, external $Ba^{2+}$ at concentrations ranging from 25 to 100 μM caused a slow channel block, consistent with the binding of a single $Ba^{2+}$ ion at a site located at half the membrane span. It is concluded on the basis of these observations that HeLa cells expressed a Kir2.1 type inwardly rectifying channel likely to be involved in maintaining and regulating the cell resting potential.

**Key words:** HeLa cells — Inward rectifier — Cloning — Kir2.1 — *Xenopus* oocyte — Channel

## Introduction

There is now increasing evidence that potassium channels play a prominent role in a large variety of cellular events including regulation of membrane potential, $Ca^{2+}$ signaling, cell proliferation and regulation of cell volume. Numerous studies have established for instance, that the activation of $Ca^{2+}$-dependent $K^+$ channels was essential to provide or maintain a sufficient driving force for $Ca^{2+}$ entry in cells stimulated with agonists linked to the phosphoinositide pathway [30]. Similarly, potassium channel antagonists have been reported to inhibit the proliferation of many cell types including rapidly dividing tumor cells [42]. The cellular mechanism by which $K^+$ channel activation is related to cell proliferation remains however largely undetermined. In the particular case of HeLa cells, a cell line derived from an epidermoid carcinoma of the cervix, two distinct $K^+$ permeable channels have been so far identified: a $Ca^{2+}$-activated $K^+$ channel of intermediate conductance (IK($Ca^{2+}$)) the activity of which appeared voltage-insensitive, and a $Ca^{2+}$-

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independent inwardly rectifying $K^+$ channel [6, 31, 33, 34]. Previous studies have shown that the IK($Ca^{2+}$) channels in these cells are responsible for the variations in membrane potential resulting from transient fluctuations in internal $Ca^{2+}$ triggered by histamine [31, 33]. This channel has been extensively characterized both at the single channel and whole cell levels [6, 33].

Single channel evidence was also provided for a strong inwardly rectifying $K^+$ channel in resting HeLa cells. The channel conductance for inward currents was estimated at 40 pS and the channel open probability was found to vary as a function of the external potassium concentration while being insensitive to internal $Ca^{2+}$ [32, 34]. The channel rectification was strong enough to allow large inward $K^+$ currents at potentials negative to $E_K$, with little or no outward currents. Inwardly rectifying channels with similar current/voltage characteristics have already been reported in a large variety of cellular preparations [18, 20, 21, 39]. In addition, recent observations have indicated that the inward rectification in these cases was resulting from an internal block of the channel by $Mg^{2+}$ and/or polyamines such as spermine or spermidine [8, 17, 35, 41]. Because of their non-ohmic conductance properties, IRK1 channels have often been involved in the maintenance of cell resting potential. In fact, it was suggested that the role of inwardly rectifying channels in nonexcitable cells was to set and stabilize membrane potential at rest, while providing targets for a cellular control of plasma membrane $K^+$ permeability via G protein linked processes [22]. Because of their function as cell membrane regulators, it was also argued that inward rectifiers could be involved in cell-cycle progression [15].

Despite their potential contribution to various cellular events, little concern has been given so far to the $Ca^{2+}$-insensitive inwardly rectifying $K^+$ channels in HeLa cells. A study was therefore undertaken aimed at determining to what extent the inward rectifying $K^+$ channel present in these cells corresponds to the IRK1 type inward rectifier observed in several excitable and nonexcitable cell preparations [18, 20, 21, 39]. The molecular nature of the inward rectifier was established by RT-PCR based cloning and confirmed by sequencing and functional expression of the cloned channel in *Xenopus* oocytes. Our results show that HeLa cells express a 40 pS IRK1 channel sensitive to external $Cs^+$ and $Ba^{2+}$, which is identical to the human Kir2.1 channel cloned from human heart [25, 43], brain [1] and lens epithelium cells.

## Materials and Methods

### cDNA Cloning and Sequencing

#### *cDNA Cloning*

The cloning of the HeLa cell inwardly rectifying channel was carried out by RT-PCR, using two oligonucleotides designed from the previously published human heart IRK1 channel sequence [25]. The rationale for choosing IRK1 was based essentially on the similarities observed between the inward rectifier current/voltage curves measured in HeLa cells, and the reported current/voltage characteristics for the human cardiac IRK1 channel. These two oligonucleotides (base pairs (bp) 311–349 and 1584–1622) were chosen to yield a 1311 bp fragment containing the entire coding sequence of the IRK1 channel. Total RNA was extracted from HeLa cells using the Trizol reagent kit (Gibco-BRL, Gaithersburg, MD) and poly $(A)^+$ RNA was purified on oligo(dT) cellulose columns. One μg of HeLa poly$(A)^+$ RNA was then reverse transcribed with 200 U of SuperScript II reverse transcriptase (Gibco-BRL) and 2 pmoles of the 3′-end gene specific primer (AGG AAT CAG TCA GTC ATA TCT CCG ACT CTC GCC CTA AGG) for 50 min at 42°C. One tenth of this cDNA was directly used as a template for PCR amplification, using the 5′-gene specific primer (TCC CCA GCA GAA GCG ATG GGC AGT GTG CGA ACC AAC CGC) and 2.5 U of Pfu DNA Polymerase (Strategene, La Jolla, CA). PCR was performed as follows: 1 cycle (95°C, 1 min), 30 cycles (95°C, 15 sec; 60°C, 30 sec and 72°C, 90 sec), followed by a final extension for 10 min at 72°C. A PCR-fragment of the expected size of 1311 bp was obtained, gel-purified and subcloned into pGEM-T (Promega, Biotech). The full length IRK1 cDNA was then subcloned into the mammalian expression vector pMT21.

#### *DNA Sequencing*

The cDNA cloned in pMT21 plasmid was sequenced by the dideoxynucleotide chain termination method using the T7 Sequenase kit (USB, Cleveland, OH) with synthetic oligonucleotides as primers. Sequence comparisons against databases and other sequence analyses were carried out using softwares available at Infobiogen (www.infobiogen.fr).

### Northern Blot Analysis

For Northern blot analysis, total RNA (20 μg), was prepared from the indicated cells using the Trizol reagent kit (Gibco-BRL, Gaithersburg, MD), and separated by electrophoresis on a formaldehyde-agarose (1.2%) gel. RNA was transferred onto a nylon membrane (Boehringer Mannheim) by capillary transfer and fixed to the membrane by UV irradiation. The membrane was then hybridized according to the method of Church and Gilbert (1984) [3] to a $^{32}$P-labeled β-actin or entire IRK1 cDNA probe (Oligolabeling kit, Pharmacia Biotech) of 1–2 $10^9$ dpm/μg specific activity. After overnight hybridization at 68°C, blots were washed twice at high stringency (1% SDS, 40 mM $NaH_2PO_4$ pH 7, 1 mM EDTA at 65°C during 20 min) and exposed to Kodak XAR film at −70°C for 2–3 days.

### Cell Cultures

HeLa cells were grown on 2.5 cm microscope slides to accommodate the perfusion chamber used for patch-clamp experiments. The cells were used 5–7 days after being subcultured. The culture medium was a minimum essential medium Earle's base (Gibco-BRL) with 25 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) and 6 mM bicarbonate at pH 7.4. The medium was supplemented with 10% fetal bovine serum (Gibco-BRL) and 10 mg/l of gentamycin. For Northern blot analysis, bovine aortic endothelial (BAE) cells were grown in low-glucose Dulbecco's modified Eagle Medium (D-MEM; Gibco) containing 10% new born calf serum, 3.7 g/l $NaHCO_3$, 100 U/ml penicillin and 100 μg/ml streptomycin in humidified air with 5% $CO_2$ atmosphere at 37°C. Similarly, WI-38 cells were cultured in low-glucose D-MEM (Gibco) with 10% foetal bovine serum (FBS) plus 3.7 g/l $NaHCO_3$ in 5% $CO_2$ at 37°C. The FBS in this case was not heat

inactivated. ECV304 were grown in a M199 medium (Gibco) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 10% FBS and 2.2 g/l $NaHCO_3$ in a $CO_2$ atmosphere as described previously.

### Oocytes

Mature oocytes (stage V or VI) were obtained from *Xenopus laevis* frogs anesthetized with 3-aminobenzoic acid ethyl ester. The follicular layer was removed by incubating the oocytes in a $Ca^{2+}$-free Barth's solution containing collagenase (1.6 mg/ml, Sigma) for 45 min. The composition of the Barth's solution was (in mM): 88 NaCl, 3 KCl, 0.82 $MgSO_4$, 0.41 $CaCl_2$, 0.33 $Ca(NO_3)_2$ and 5 HEPES (pH 7.6). Defolliculated oocytes were stored at 18°C in Barth's solution supplemented with 5% horse serum, 2.5 mM Na pyruvate, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Oocytes were studied 3–5 days after co-injection of 0.92 ng of the IRK1 cDNA cloned in pMT21 and 1.38 ng of cDNA coding for a green fluorescent protein that was used as a marker for nuclear injection [4].

Prior to patch-clamping, defolliculated oocytes were kept in a hypertonic solution containing (in mM) 250 KCl, 1 $MgSO_4$, 1 EGTA, 50 sucrose and 10 HEPES buffered at pH 7.4 with KOH. The vitelline membrane was then peeled off using forceps, and the oocyte was transferred to a superfusion chamber for patch-clamp measurements.

### Patch-Clamp Recording

Single channel recordings were carried out either in the cell-attached or inside-out patch-clamp configuration using an Axopatch 200A amplifier (Axon Instruments). Patch pipettes were pulled from Pyrex capillaries (Corning 7040) using a David Kopf programmable pipette puller (Model 750) and used uncoated. The resistance of the patch electrodes ranged from 4 to 10 MΩ. Unless specified otherwise, the membrane potential in the cell-attached configuration is expressed as $-V_p$, where $V_p$ is the pipette applied potential. Current traces were recorded using a Racal FM tape recorder at a bandwidth of 1.25 kHz (Racal). For offline analysis, the signal was sampled at 1.0 kHz and filtered at 500 Hz with two low-pass four-pole Bessel filters (VVS 300B, Frequency Devices, Haverhill, MA) connected in series. For the $Cs^+$ block experiments, current records were filtered at 1 kHz and sampled at 5 kHz. Base line drift was corrected through a multiple linear interpolation procedure. When required, the open channel probability, $P_o$, was estimated from current amplitude histograms on the basis of a binomial distribution as described elsewhere [19]. The stationarity of the recorded signal was tested according to the criteria defined in Denicourt et al. [5]. The voltage dependent reduction of $P_o$ caused by $Cs^+$ and $Ba^{2+}$ was fitted to

$$P_o = \frac{1}{1 + exp\,\dfrac{\delta Zq(V - V_0)}{KT}} \qquad (1)$$

where $V_0$ is the voltage for half reduction, δ the fractional electrical distance of the blocker binding site, $Z$ the valence of the blocking agent, $q$, the elementary charge, $K$ and $T$ the Boltzmann's constant and the temperature respectively. All the experiments were performed at room temperature (24°C).

### Solutions

The solution referred to as 200 $K_2SO_4$ had a composition as follows (in mM): 200 $K_2SO_4$, 1.8 $MgCl_2$, 10 Hepes buffered with KOH at pH 7.4. Sulfate salts were used to avoid detection of endogenous chloride channels. Solutions with reduced $K_2SO_4$ concentrations were obtained by isoosmolar substitution of $K_2SO_4$ by $Na_2SO_4$. The $K^+$ ion activity coefficients for the various $K_2SO_4$ solutions were obtained from the literature [27]. For patch-clamp experiments carried out in the presence of $Ba^{2+}$, we used a 200 mM KCl pipette solution containing (in mM): 200 KCl, 1.8 $MgCl_2$, 1 EGTA buffered at pH 7.4 with 10 HEPES and KOH.

## Results

### Primary Structure of IRK1 in HeLa Cells

Analysis of the nucleotide sequence of the coding region for the HeLa IRK1 channel revealed perfect identity to the human cardiac channel coded by the HH-IRK1 gene (GenBank U12507) [25]. A near perfect identity was also obtained when compared to the nucleotide sequences reported for the IRK1 channels in two nonexcitable human cell preparations, namely lens epithelial cells (GenBank AF021139) and blood eosinophils (GenBank AF011904) [38], with sequences differing by one and two nucleotides, respectively. The amino acid sequence was found to be 100% homologous to the human IRK1 channel cloned either from heart (GenBank U16861) [25, 43], brain (GenBank U22413) [1] or lens epithelium (GenBank AF021139), and more than 99% homologous to the IRK1 channels from human blood eosinophils [38], bovine aortic endothelial cells (GenBank U95369) [9] and mouse macrophage (GenBank X73052) [14].

### Distribution of HeLa IRK1

A Northern blot analysis was also performed to determine if the IRK1 channel from HeLa cells is equally expressed in other nonexcitable cell preparations, including endothelial cells. The results illustrated in Fig. 1 provide evidence for a strong signal at ~5.3 kb in HeLa cells, while indicating a transcript of the same size in WI-38 cells, a human diploid cell line from normal embryonic lung tissue, in ECV304 cells, a cell line derived from a human umbilical vein, and in BAE cells. The rank order intensity for the 5.3 kb signal relative to β-actin yielded HeLa > WI-38 ≅ ECV304 > BAE, with HeLa cells expressing the highest relative level of IRK1 mRNA compared to the other cell types investigated. The resulting molecular weight was found to be in agreement with the observations reported by Forsyth et al. [9] for the size of the BAE inward rectifier mRNA, but appeared to differ from the 2.7 kb molecular weight measured for the human Kir2.3 channel mRNA [24, 37]. Similarly, our results are not compatible with the 2.4 kb and 4.4 kb signals associated to the Kir2.2 inward rectifier obtained from rat brain [13]. These observations together with the identity we found between the HeLa IRK1 nucleotide sequences and the sequence of the human cardiac IRK1 channel, argue for the presence in

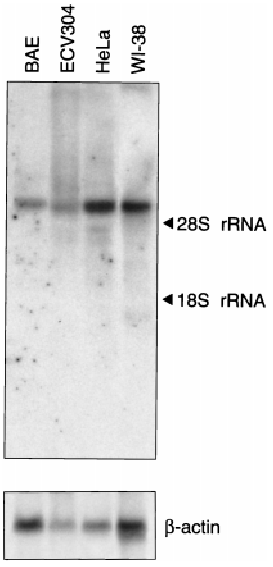


**Fig. 1.** Northern blot analysis of IRK1 in nonexcitable cells. Twenty (20) μg of total RNA from BAE, ECV304, HeLa and WI-38 cells were separated by electrophoresis through a 1.2% agarose-formaldehyde gel and hybridized with the entire $^{32}$P-labeled IRK1cDNA (*see* Materials and Methods). A transcript of approximately 5.3 kb was present in each of these cell preparations. The quantity of RNA in each sample was standardized using a $^{32}$P-labeled β-actin probe. The most important signal relative to β-actin was detected with mRNA from HeLa cells.

HeLa, BAE, ECV304 and WI-38 cells of an inward rectifier most likely of the Kir2.1 family, while providing evidence against the expression in these cells of Kir2.2 and Kir2.3 inwardly rectifying channels.

### IRK1 in HeLa Cells

The presence of a strong inwardly rectifying $K^+$ channel in HeLa cells was confirmed with a series of cell-attached patch-clamp experiments in which both the patch electrode and the cell bathing medium contained 200 mM $K_2SO_4$ in low-$Ca^{2+}$ conditions (0.35 μM). Examples of the resulting single channel records are presented in Fig. 2*A*. Despite clear channel openings at negative applied potentials, there were no detectable outward current jumps at positive potential values ($-V_p$), indicating a strong inwardly rectifying current/voltage relationship. In addition, the recorded channel showed a slow fluctuation pattern, distinct from the rapid current fluctuations which characterize the $Ca^{2+}$-activated potassium channel of intermediate conductance also present in HeLa cells [33, 34]. The channel current/voltage relationship is illustrated in Fig. 2*B*. The slope conductance measured for inward currents within the voltage range −170 to −100 mV was estimated at 42 ± 1 pS ($n = 3$), but decreases to 33 ± 2 pS ($n = 6$) for voltages ranging from −100 to −40 mV. Excised inside-out experiments confirmed in addition that channel gating was independent of the cytosolic $Ca^{2+}$ concentration (*data not shown*).

### Functional Expression of the IRK1 Channel in Oocytes

To confirm that the channel cloned from HeLa cells corresponded to a Kir2.1 type inward rectifier, a series of patch-clamp experiments was performed either in the cell-attached or inside-out configuration on *Xenopus* oocytes injected with HeLa inward rectifier cDNA as described in Materials and Methods. Single channel events characteristic of IRK1 were observed in nearly 100% of the eggs expressing the green fluorescent protein. Representative cell-attached single channel records obtained at potentials ranging from −120 to 120 mV are illustrated in Fig. 3*A*. In these experiments, both the pipette and external bathing medium contained the 200 mM $K_2SO_4$ solution. The current traces in Fig. 3*A* provide clear evidence for a strong inwardly rectifying behavior, with channel openings detected at negative membrane potential values exclusively. The channel unitary conductance for inward currents was equal to 40 ± 1 pS ($n = 3$) and to 30.8 pS ± 2.8 ($n = 6$) (Fig. 4*A*) for applied voltages ranging from −100 to −160 mV and from −40 to −100 mV respectively. These values are in accordance with the results obtained for the endogenous inward rectifier in HeLa cells (Fig. 2). No channel with similar inward rectifying properties could be detected in control experiments ($n = 40$) carried out on *Xenopus* oocytes injected with a cDNA-free carrier solution. In addition, the channel showed a slow fluctuation pattern typical of the inward rectifying $K^+$ channel detected in HeLa cells. Figure 3*B* presents the results of an inside-out single-channel experiment performed in symmetrical 200 mM $K_2SO_4$ + 1.8 mM $MgCl_2$ conditions. Aside from being noisier at positive applied membrane potentials, the resulting current traces were identical to those measured in the cell-attached configuration. The channel was clearly inwardly rectifying, with no detectable current jumps at positive potentials up to +100 mV. Although cell-attached patches usually displayed 5 to 10 channels, most inside-out experiments showed a reduced single-channel activity. This phenomenon is likely to be related to a rundown of the channels in patch excised experiments. Current/voltage curves measured in cell-attached experiments with pipettes containing (in mM): 200, 100, 50, and 25 $K_2SO_4$ are presented in Fig. 4*A*. The bathing medium was a 200 mM $K_2SO_4$ solution as described previously. Decreasing the pipette $K^+$ concentration from 200 mM $K_2SO_4$ to 25 mM $K_2SO_4$ resulted in a negative shift of the extrapolated reversal potential value by 40 mV. The observed shift is close to 38 mV, the expected variation in zero current potential for a highly $K^+$-selective channel, when taking into account the activity coefficients for $K^+$ in 200 mM and 25 mM $K_2SO_4$

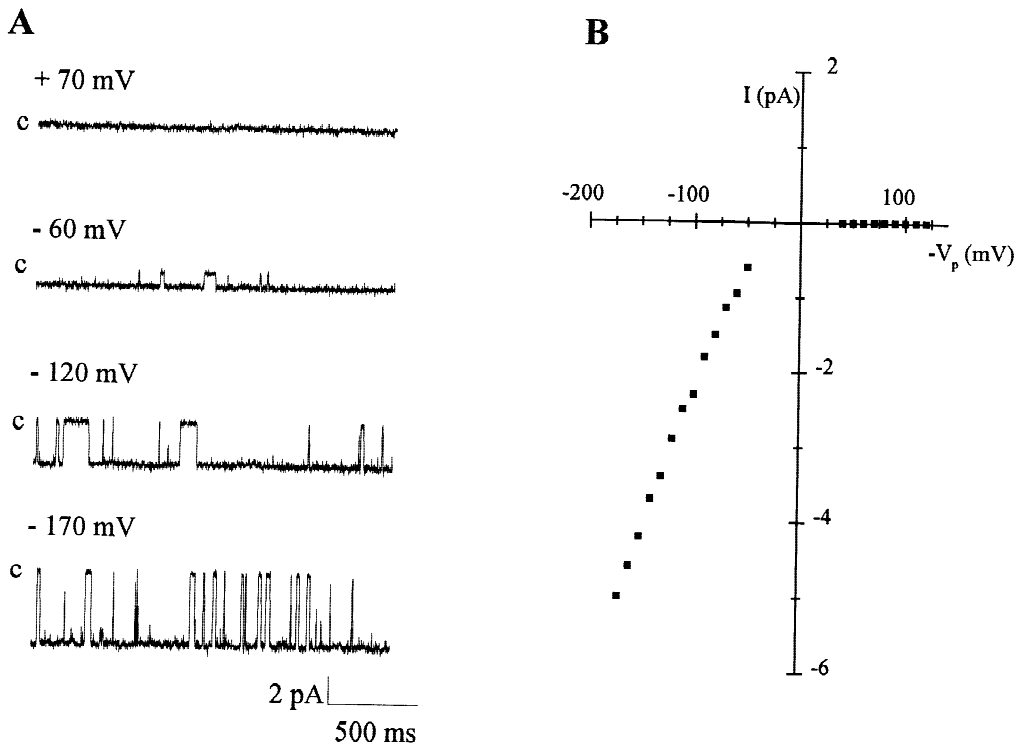


**Fig. 2.** Evidence of IRK1 type channels in HeLa cells. (*A*) Cell-attached single channel recordings of IRK1 channels measured at $-V_p$, where $V_p$ is the pipette applied potential, ranging from 70 to −170 mV. The patch electrode and cell bathing medium contained 200 mM $K_2SO_4$ in low free $Ca^{2+}$ conditions (0.35 μM). There were no detectable outward currents under these conditions indicating a channel with a strong inwardly rectifying behavior. Current traces were filtered at 250 Hz. The letter *c* indicates the closed-state current level. (*B*) Current/voltage relationship of the channel illustrated in *A*. The slope conductance for inward currents within the voltage range −170 to −100 mV was estimated at 42 ± 1 pS ($n = 3$), but decreases to 33 ± 2 pS ($n = 6$) for voltages ranging from −100 to −40 mV.

respectively. More importantly, inward rectification was found to depend on the $K^+$ concentration in the patch electrode, with no detectable outward currents at potentials positive to $E_K$. The results in Fig. 4*A* therefore provide clear evidence for the expression of a Kir2.1 type inward rectifier. The variation in unitary conductance, Γ, as a function of $\alpha_K$, the external $K^+$ ion activity, is presented in Fig. 4*B*. The continuous curve was computed according to $\Gamma = \Gamma_0 [\alpha_K]^\beta$ with $\Gamma_0 = 3.3$ pS and $\beta = 0.5$. These results confirm the square root dependency of channel conductance on the external $K^+$ concentration as already documented in several studies on strong inwardly rectifying channels [10, 36]. In addition, it is concluded on the basis of the data presented in Fig. 4*B* that the value of Γ under normal physiological external $K^+$ conditions should equal approximately 7.4 pS.

### Block by $Cs^+$ and $Ba^{2+}$

One of the main characteristic features of inward rectifiers is their susceptibility to blockade of the channel pore by external $Cs^+$ and $Ba^{2+}$. Figure 5*A* shows an example of cell-attached single channel recordings obtained with 500 μM $Cs^+$ added to the 200 $K_2SO_4$ solution in the patch electrode. The presence of $Cs^+$ caused a voltage-dependent flickering, typical of a fast channel blocking process. Compared to control measurements (Fig. 3*A*), there was a clear reduction of $P_o$ at increasing negative membrane potential values. These measurements were limited, however, to potentials more negative than −30 mV, due to the absence of detectable current jumps at positive potential values. The voltage dependence of the $Cs^+$ block was quantified by fitting the experimental data to Eq. (1). Figure 5*B* summarizes the results obtained from seven different experiments. The continuous curve was computed from Eq. (1) with $Z\delta = 2$ and $V_0 = -92$ mV. A value of δ greater than 1 in this case is consistent with a channel accommodating multiple ions, thus supporting the results presented in Fig. 4*B* where the nonlinear dependence of conductance on $K^+$ activity indicated a deviation of the independence principle and possible interactions between $K^+$ ions. A more detailed analysis of the $Cs^+$ block mechanism was carried out in a few recordings where a single channel was present. An example of this type of analysis is presented in Fig. 5*C*, where the variation in transition rate between the open and closed states during $Cs^+$ block was plotted as a function of voltage. The results obtained indicate

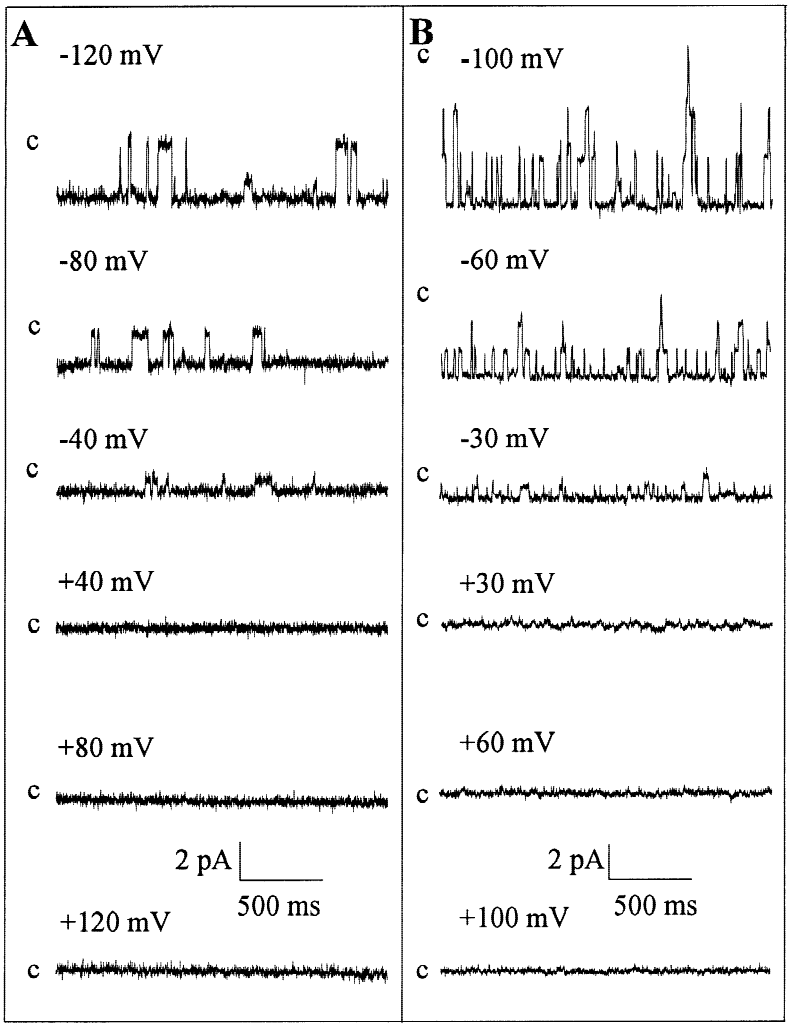


**Fig. 3.** Functional expression of the HeLa IRK1 channel in *Xenopus* oocytes. (*A*) Examples of cell-attached single channel recordings obtained with 200 $K_2SO_4$ in both the patch pipette and external bathing medium. Experiments carried out on an oocyte injected with 0.92 ng of HeLa IRK1 cDNA cloned in pMT21. The inwardly rectifying behavior is confirmed by the absence of outward currents at positive $-V_p$ values. (*B*) Examples of inside-out single channel recordings measured in 200 mM $K_2SO_4$ + 1.8 mM $MgCl_2$ symmetrical conditions. Current fluctuations with multiple channel openings can readily be observed at negative potentials, whereas no current jump could be measured at positive potential values. The slope conductance for inward currents was estimated in both cases at 40 ± 1 pS ($n = 3$) and to 30.8 ± 2.8 pS ($n = 6$) for applied voltages ranging from −100 to −160 mV and from −40 to −100 mV respectively. Current traces were filtered at 250 Hz. The letter *c* indicates the closed-state current level.

that the number of transition/s from the closed to open state was independent of voltage, whereas the transition rate from the open to closed state increased as a single exponential at negative potentials with an e-fold variation/22 mV. On the basis of these observations, it was concluded that $Cs^+$ exits from its binding site in a voltage-independent manner, and that the $Cs^+$ blocking process involves more than one ion.

Channel block was also observed when $Ba^{2+}$ was added to the pipette filling solution. Figure 6*A* shows cell-attached current traces obtained with a patch electrode containing 100 μM $Ba^{2+}$ + 200 mM KCl. In contrast to $Cs^+$, the presence of external $Ba^{2+}$ initiated the appearance of long channel closures in a voltage-dependent manner. There was no significant change in the channel unitary current amplitude, indicating a slow voltage-dependent blocking process. The effect of $Ba^{2+}$ was quantified by measuring $P_o$ as a function of voltage. The results of this analysis are presented in Fig. 6*B*. There was a clear decrease of $P_o$ at increasing negative potential values, with total inhibition of channel activity at −120 mV in the presence of 100 μM external $Ba^{2+}$. Decreasing the external $Ba^{2+}$ concentration to 25 μM shifted the voltage dependence of the $Ba^{2+}$ block 30 mV to the left. In addition, analysis of the voltage dependence of $P_o$ using equation (1) led to $\delta = 0.5$, indicating that the binding site for $Ba^{2+}$ block was located half-way across the membrane span. These results confirm the presence in HeLa cells of an inwardly rectifying channel characterized by a high sensitivity to both $Cs^+$ and $Ba^{2+}$ ions.

## Discussion

This work provides the first molecular evidence for the presence of a Kir2.1 inward rectifying $K^+$ channel in HeLa cells, a human cell line derived from an epidermoid carcinoma of the cervix. The amino acid sequence derived for the HeLa inwardly rectifying channel ap-

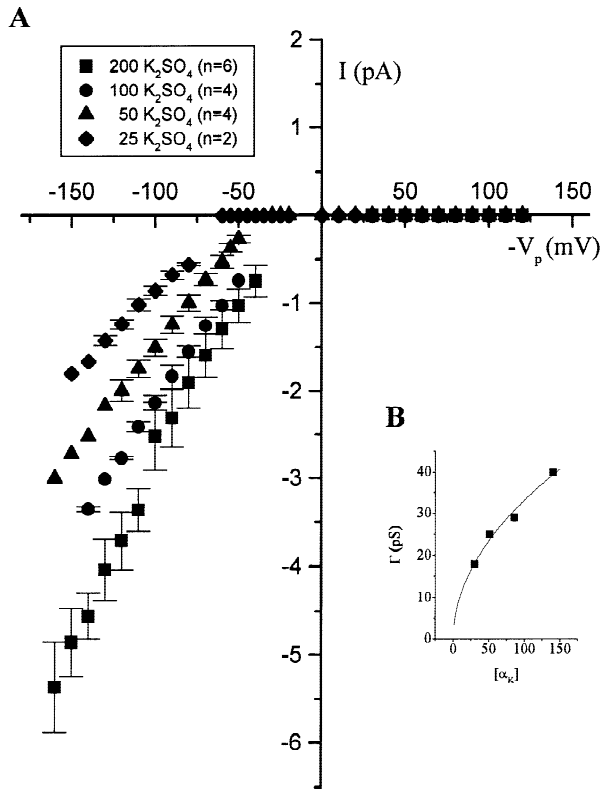


**Fig. 4.** (*A*) Current/voltage properties of the HeLa IRK1 expressed in *Xenopus* oocytes. Data were obtained from cell-attached experiments in which oocytes were bathed in a $Ca^{2+}$-free (0.35 μM) 200 mM $K_2SO_4$ external medium with pipettes containing $K_2SO_4$ at concentrations ranging from 25 to 200 mM. Solutions with reduced $K_2SO_4$ concentrations were obtained by isoosmolar substitution of $K_2SO_4$ by $Na_2SO_4$. These results indicate that the inward rectification varies according to the Nernst equilibrium potential for $K^+$. The relationship between the channel slope conductance for inward currents, Γ, and the activity of $K^+$ ions in the pipette, $\alpha_K$, is illustrated in *B*. The continuous line was computed according to $\Gamma = \Gamma_0 [\alpha_K]^\beta$ with $\Gamma_0 = 3.3$ pS and $\beta = 0.5$, confirming the square root dependency of Γ as a function of $\alpha_K$.

peared identical to the sequences reported for the human Kir2.1 channels obtained from heart [25, 43], brain [1] or lens epithelium. There was a two amino acid difference when compared to the Kir2.1 channel from human blood eosinophils [38]. Our results show, in addition, that the same current/voltage properties are shared by both the Kir2.1 channel cloned from HeLa cells and the endogenous HeLa inward rectifier (Fig. 2). The unitary conductance was equal to 40 pS for inward currents within the voltage range −100 to −160 mV in both cases, and there were no detectable outward currents at potentials positive to $E_K$. IRK1 type inward rectifiers of smaller unitary conductance were never observed in HeLa cells, despite clear indications for the presence of a subconductance level equal to 75% of the full open state [32]. This is in contrast with other reports on IRK1 channels describing the occurrence of four subconductance levels of 7 pS in 140 mM KCl conditions [28]. However, transitions to subconducting states were more frequent in IRK1 expressed in *Xenopus* oocytes than in HeLa cells, suggesting the contribution of cell specific factors to channel overall kinetic behavior. Taken together, these results confirm, as we suggested previously [32, 34], that the inward rectifying channel in HeLa cells corresponds, both structurally and functionally, to the IRK1 reported in several human excitable cells [1, 25, 43]. In support of this proposal, the northern blot analysis illustrated in Fig. 1 showed that the size of the HeLa IRK1 mRNA is compatible with the molecular weight reported for the human Kir2.1 channel transcript, while providing evidence against the expression of Kir2.2 and Kir2.3 inward rectifiers in these cells. Identical results were obtained using BAE, WI-38 and ECV304 cells, an indication that Kir2.1 may well constitute the dominant inwardly rectifying channel expressed in a large variety of nonexcitable cell preparations.

IRK1 channels are known to be highly susceptible to the blocking action of external cations such as $Cs^+$ and $Ba^{2+}$ [10, 29, 36]. The single channel recordings presented in Figs. 5 and 6 show that *Xenopus* oocytes injected with HeLa IRK1 cDNA expressed an inward rectifying channel that can be blocked by $Cs^+$ and $Ba^{2+}$ in a voltage-dependent manner at μM concentrations. The action of $Cs^+$ was typical of a fast blocking process, with frequent and short interruptions of individual channel openings resulting in an overall decrease of the channel open probability at increasing negative potential values (Fig. 5). The mean value of the channel closed time intervals was found however to be voltage independent (Fig. 5*C*), indicating that the voltage dependence of the $Cs^+$ block was coming exclusively from an effect of $Cs^+$ on the channel mean open time. Furthermore, since the curve fitting to Eq. (1) of the experimental data in Fig. 5*B* led to an equivalent valence $z\delta = 2$, the blocking action of $Cs^+$ cannot be described by the original Woodhull model [44]. In fact, this observation and the square root dependence of the inward current conductance as a function of $\alpha_K$, the $K^+$ ion activity, provide strong evidence for a channel with multiple ion occupancy. In contrast, $Ba^{2+}$ caused a slow block, compatible with a binding site located at 50% of the membrane span. The fractional electrical distance in this case appeared smaller than that obtained in embryonic *Xenopus laevis* myocytes [2], starfish eggs [10] and skeletal muscle [36] where values ranging from 0.7 to 0.8 were reported. Calculations carried out using Eq. (1) with $\delta = 0.5$ predicted nevertheless that a fourfold increase in $Ba^+$ concentration should right shift the voltage dependence of the $Ba^{2+}$ block by 34 mV. This value is in close agreement with the results presented in Fig. 6*B*, where a shift of $V_0$ from −42 to −12 mV was obtained by decreasing the $Ba^{2+}$ concentration from 25 to 100 μM. Moreover, the external $Ba^{2+}$ con-

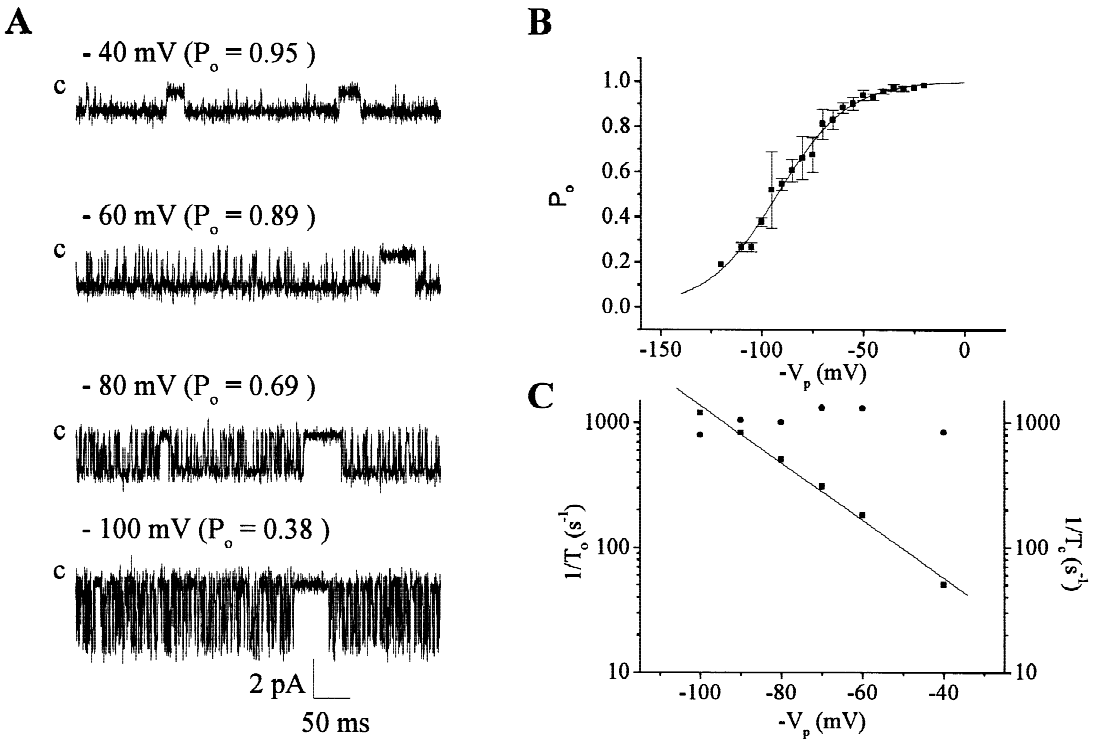


**Fig. 5.** Block of the HeLa IRK1 channel expressed in *Xenopus* oocytes by external $Cs^+$. (*A*) Examples of cell-attached recordings obtained at potentials $-V_p$ ranging from −40 to −100 mV using a patch electrode containing 200 mM KCl + 500 μM CsCl. The oocyte was bathed in a 200 mM KCl medium. Under these conditions, channel flickering increased at more negative potential values, resulting in a decrease of the channel open probability. Current records were filtered at 1 kHz and samples at 5 kHz. The letter *c* indicates the closed-state current level. (*B*) Channel open probability as a function of $-V_p$. Data were combined from 7 different experiments. The continuous curve was computed from Eq. (1) with $Z\delta = 2$ and $V_0 = -92$ mV. (*C*) Representative variation of the channel mean number of transition/s between open and closed states as a function of $-V_p$. The number of transition/s from the closed to the open state (circle) appeared voltage-independent, where as the number of transition/s from the open to closed state (square) varied exponentially with an e-fold variation per 22 mV.

centration for half inhibition was estimated at 155 and 4 μM for applied voltages of 0 and −90 mV, respectively. These observations compare favorably with the results obtained in *Xenopus laevis* myocytes [2], starfish eggs [10] and skeletal muscle [36]. A $Ba^{2+}$ concentration of less than 1 μM was reported however for half inhibition of the inwardly rectifying $K^+$ channel in coronary endothelial cells from guinea pig heart at a potential of −70 mV [40]. The sensitivity to $Ba^{2+}$ appeared in this particular case more important than that illustrated in Fig. 6.

The role of strong inward rectifying channels in non-excitable cells remains still speculative, but indirect evidence suggests that they may be involved in the stabilization and regulation of the membrane potential in resting cells [22] and in cell proliferation [15]. HeLa cells have also been shown to express a calcium-activated $K^+$ channel of intermediate conductance IK($Ca^{2+}$) [33]. This channel is likely to be responsible for the hyperpolarizing response triggered by external agonist linked to the phosphoinositol pathway [34]. RT-PCR based cloning experiments from this laboratory have confirmed that the IK($Ca^{2+}$) channel in HeLa cells corresponds in fact to the IK($Ca^{2+}$) channels recently cloned from human T lymphocytes (hKCa4) [16] and human pancreas (hIK1) [12] (*data not shown*). In addition, preliminary PCR evidence by Riquelme et al. [26] has indicated the presence in HeLa cells of a $Ca^{2+}$-activated channel of the SK family. A molecular characterization in these cells of SK channels is, however, still lacking. Finally, evidence was provided for a volume activated $Cl^-$ channel in HeLa cells [7]. It follows that the regulation of the membrane potential at rest is likely to involve each of these ion channels to various degrees. The exact contribution of the inwardly rectifying channel to the cell current/voltage properties at rest may thus be variable as reported for endothelial cells, where high, medium and low IRK1/$Cl^-$ conductance ratios were observed [22].

The relative expression of IRK1 compared to IK($Ca^{2+}$) channels has been linked to cell proliferation in many cases [11, 23]. For instance the work of Pena and Rane [23] on the myogenic cell line C3H10T1/2 −MRF4 has shown that a downregulation of the IK($Ca^{2+}$) channels in this case leads to an increased expression of IRK1

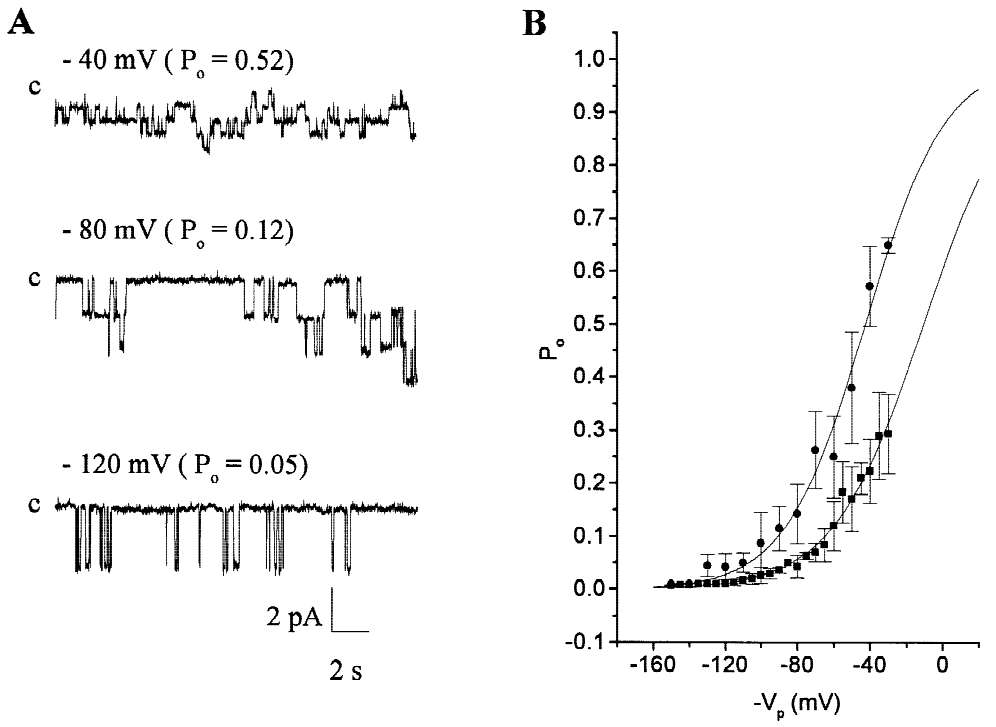


**Fig. 6.** Block of the HeLa IRK1 channel expressed in *Xenopus* oocytes by external $Ba^{2+}$. (*A*) Representative cell-attached recordings obtained at potentials $-V_p$ ranging from −40 to −120 mV using a patch electrode containing 200 mM KCl + 100 μM $BaCl_2$. The oocyte was bathed in a 200 mM KCl medium. Under these conditions, $Ba^{2+}$ caused a slow blockade of the channel, resulting in a decrease of the channel open probability. Current traces were filtered at 250 Hz. The letter *c* indicates the closed-state current level. (*B*) Voltage dependency of the channel open probability as a function of $-V_p$ measured at 100 μM (square, $n = 3$) and 25 μM (circle, $n = 4$) external $Ba^{2+}$, respectively. The continuous curves were computed from Eq. (1) with $\delta = 0.5$ with $V_0 = -42$ and −12 mV for external $Ba^{2+}$ concentrations of 25 μM and 100 μM, respectively.

whereas an overexpression of IK($Ca^{2+}$) causes an IRK1 down regulation. The possibility that a similar mechanism may be operational in HeLa cells needs further investigations. Similarly, the presence of a $Ca^{2+}$-insensitive inwardly rectifying $K^+$ channel and of IK($Ca^{2+}$) channels has also been reported by Lepple-Wienhues et al. [15] in SK MEL 28, a cell line established from a human cutaneous melanoma. The inward rectifier appeared responsible in this case for most of the resting cell $K^+$ permeability. In addition, their results indicated that cell proliferation could be inhibited by agents such as $Ba^{2+}$ and quinidine which blocked the inward rectifier, but remained insensitive to charybdotoxin an IK($Ca^{2+}$) blocking agent. It was concluded that the inward rectifying channels in this case could modulate cell-cycle progression by an action on membrane potential which in turn can affect the driving force for $Ca^{2+}$ influx [15]. The exact molecular nature of the inward rectifier in SK MEL 28 cells is not, however, currently known. Such a behavior may prevail in cell type such as HeLa cells which are mitogenically very active. How IK($Ca^{2+}$) can modulate IRK1 expression and the relative contribution of each channel to cell proliferation remains to be established.

## Conclusion

We have cloned and functionally expressed an inwardly rectifying channel of the Kir2.1 subfamily from HeLa cells. This channel showed features characteristic of the IRK1 type channel identified in several excitable cell preparations. It is likely that this channel serves as a membrane potential regulator under normal physiological conditions.

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