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TOPICAL REVIEW

Intracellular BK_{Ca} (iBK_{Ca}) channels

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Abstract The large conductance calcium- and voltage-activated potassium channel (BK_{Ca}) is widely expressed at the plasma membrane. This channel is involved in a variety of fundamental cellular functions including excitability, smooth muscle contractility, and Ca^{2+} homeostasis, as well as in pathological situations like proinflammatory responses in rheumatoid arthritis, and cancer cell proliferation. Immunochemical, biochemical and pharmacological studies from over a decade have intermittently shown the presence of BK_{Ca} in intracellular organelles. To date, intracellular BK_{Ca} (iBK_{Ca}) has been localized in the mitochondria, endoplasmic reticulum, nucleus and Golgi apparatus but its functional role remains largely unknown except for the mitochondrial BK_{Ca} whose opening is thought to play a role in protecting the heart from ischaemic injury. In the nucleus, pharmacology suggests a role in regulating nuclear Ca^{2+}, membrane potential and eNOS expression. Establishing the molecular correlates of iBK_{Ca}, the mechanisms defining iBK_{Ca} organelle-specific targeting, and their modulation are challenging questions. This review summarizes iBK_{Ca} channels, their possible functions, and efforts to identify their molecular correlates.

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Introduction

Ion channels are present at the plasma membrane and in all intracellular organelles including mitochondria (O'Rourke, 2007), nucleus (Mazzanti *et al.* 1990; Singh, 2010), Golgi complex (Thompson *et al.* 2002) and endoplasmic reticulum (ER) (Osman *et al.* 2003; Ashrafpour *et al.* 2008). In intracellular organelles, they modulate the concentration of ions and play important roles in physiological events such as the voltage-dependent anion channel (VDAC) in apoptosis (Chacko *et al.* 2010), Ca^{2+}-release-activated Ca^{2+} channels (CRACs) in Ca^{2+} signalling (Yeromin *et al.* 2006), and mitochondrial K^+

Harpreet Singh (left) is Research Assistant Professor at UCLA. He obtained his PhD from the University of Edinburgh with Dr Richard Ashley and Prof. Michael Cousin, and pursued his postdoctoral training at UCLA on intracellular BK_{Ca} channels with Prof. Toro. His research focuses on the cell biology of intracellular ion channels and their role in cardiac function. He developed an interest in superresolution microscopy while working with Prof. Stefani. In 2011, Harpreet was awarded the National Scientist Development Grant by the American Heart Association. **Enrico Stefani** (middle) obtained his MD from University of Buenos Aires and a PhD from University College, London. He is Distinguished Professor of Anesthesiology and Physiology at UCLA, and John Bartley Dillon Endowed Chair in Anesthesiology. With 254 publications, chapters and reviews in the biophysics field, he is now developing superresolution fluorescence microscopy that can reach a resolution of 20–40 nm in biological samples. His custom-built state-of-the-art microscope is shared with the academic community facilitating its reproduction (http://www.anes.ucla.edu/sted/index.html). **Ligia Toro** (right) received her PhD in Physiology and Biophysics from the Centro de Investigación y Estudios Avanzados del IPN, Mexico, and postdoctoral training at Baylor College of Medicine, Houston, TX, USA. She is Professor of Anesthesiology and of Molecular & Medical Pharmacology at UCLA, and has 126 publications, reviews and book chapters with the main focus on the biology of BK_{Ca} channels. Her current interests include mitochondrial BK_{Ca} channels in the healthy and failing heart, and BK_{Ca} channel interactions with angiotensin II receptors in the kidney.

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channels in cardioprotection (Xu *et al.* 2002). In this review, we will particularly address the large conductance calcium- and voltage-activated K^+ channel (BK_{Ca}) found intracellularly but will first discuss some general properties that may define its intracellular targeting.

BK_{Ca} channels are ubiquitously expressed at the plasma membrane of nervous and non-nervous cells including smooth muscle, sensory and epithelial cells where they couple membrane potential and intracellular calcium concentration. An interesting exception is the adult cardiomyocyte which lacks BK_{Ca} at the cell surface but expresses intracellular BK_{Ca} (iBK_{Ca}) particularly in the mitochondria.

The α-subunit of BK_{Ca} channel is encoded by a single gene *Kcnma1* or *Slo1* that undergoes extensive pre-mRNA splicing (Butler *et al.* 1993). Four α-subunits assemble to form a functional ion channel pore (Fig. 1). BK_{Ca} channels

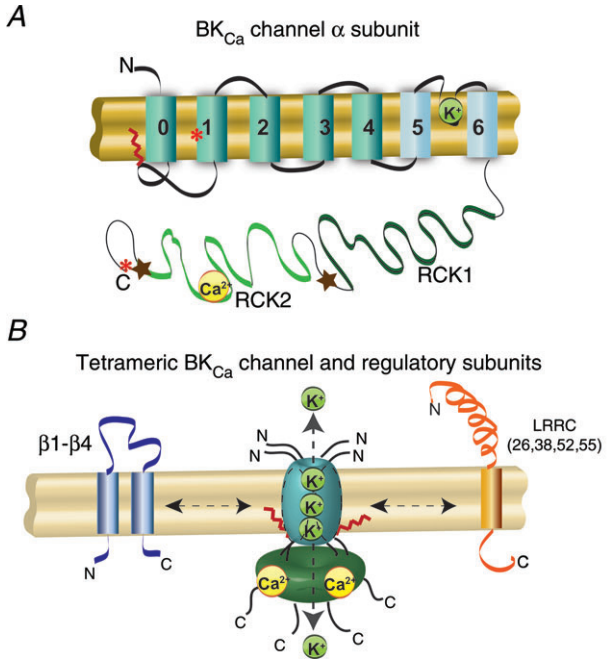

Figure 1. Topology of BK_{Ca} and modulatory subunits
A, topology of BK_{Ca} α-subunit. N-terminus and C-terminus are located in opposite sides of the membrane. At the plasma membrane the N-terminus is extracellular and the C-terminus is intracellular. Orientation in organelles is unknown. S0–S4 transmembrane domains are involved in voltage sensing. The S5–S6 linker lines the K^+-selective pore. The C-terminus has two RCK (regulator of potassium conductance) domains. RCK2 contains the Ca^{2+}-bowl. For crystallographic information see Yuan *et al.* (2010). The S0–S1 linker can be palmitoylated or myristoylated (red zig-zag line). *, sites of splice variation that can result in ER retention. Stars, sites containing export signals. *B*, diagram of BK_{Ca} channel and regulatory subunits. Four α-subunits are needed to form a functional channel. β1–β4 subunits have two transmembrane domains. N- and C-termini are facing the same side of the membrane. LRRC-subunits have a single transmembrane domain. N- and C-termini face opposite sides of the membrane.

can be in complex with several modulatory subunits with one or two transmembrane domains (Fig. 1*B*) that greatly modify the channel kinetics and voltage/Ca^{2+} sensitivities. β1–β4 have two transmembrane domains and also affect channel pharmacology and its response to lipids (Knaus *et al.* 1994; Wallner *et al.* 1999; Xia *et al.* 1999; Brenner *et al.* 2000; Meera *et al.* 2000; Uebele *et al.* 2000; Vaithianathan *et al.* 2008), while leucine-rich repeat-containing proteins (LRRC) 26, LRRC38, LRRC52 and LRRC55 are single pass membrane proteins with LRRC26 being the most potent activator producing a negative shift of ~140 mV of the voltage dependence of activation (Yan & Aldrich, 2010, 2012).

Increasing evidence suggests that splicing of BK_{Ca} α or β-subunits can govern the 'normal' traffic of the channel to the plasma membrane, consequently defining its subcellular distribution at a given time. BK_{Ca} variants originating from N- and C-terminal alternative splicing as well as C-terminal exon skipping are retained in the ER serving as repressors of BK_{Ca} channel expression at the plasmalemma (Zarei *et al.* 2004; Chen *et al.* 2005; Ma *et al.* 2007). On the other hand, β1– and β2-subunits can increase removal from the plasma membrane via endocytosis to a prelysosomal compartment (Toro *et al.* 2006; Zarei *et al.* 2007), while β4-subunits retain BK_{Ca} channels in the ER reducing its plasmalemmal localization (Shruti *et al.* 2012). Consistent with these findings, in hair cells, β1 and β4 expression reduce BK_{Ca} channels at the cell surface (Bai *et al.* 2011). Post-translational modifications can also affect the targeting of BK_{Ca} channels to the plasma membrane. Palmitoylation of intracellular loop 1 promotes cell surface expression (Jeffries *et al.* 2010), whereas internal myristoylation of loops 1 or 3 has the opposite effect (Alioua *et al.* 2011). Palmitoylation favours the exit of the channel from the ER and the *trans*-Golgi network (Tian *et al.* 2012) while myristoylation seems to favour endocytosis via clathrin-rich compartments (Alioua *et al.* 2011).

Most of the above studies have been performed in heterologous expression systems, which have been valuable in allowing the dissection of molecular mechanisms regulating the targeting of BK_{Ca} channels to the plasma membrane but only a few have been carried out in native cells. In astrocytes, transportation to the plasma membrane involves the microtubule network as fully assembled BK_{Ca} was found to be intracellularly associated with this cytoskeletal structure. When the Ca^{2+} concentration of the cytosol was elevated either pharmacologically or with thromboxane A2, iBK_{Ca} was translocated to the plasma membrane implying that microtubule-associated iBK_{Ca} was a readily available pool for astrocytes (Ou *et al.* 2009). In smooth muscle cells from pregnant mouse myometrium, iBK_{Ca} was found in the perinuclear region resulting in diminished plasma membrane expression. Possible explanations for

this phenomenon are that retention in the perinuclear region and decreased plasma membrane expression is a mechanism preparing the uterine muscle for effective contractions during delivery (Eghbali *et al.* 2003), or that in addition, iBK$_{Ca}$ within the perinuclear region serves an unknown functional role. In fibroblast-like synoviocytes from patients with rheumatoid arthritis, BK$_{Ca}$ is observed at the plasma membrane but also in the nucleus (Hu *et al.* 2012) opening the intriguing question of whether BK$_{Ca}$ localized to the nucleus may play a role in diseased states. In line with the view that iBK$_{Ca}$ channels are also targeted to intracellular organelles for a specific function – unrelated to the overall regulation of cell surface expression – in neonatal cardiomyocytes, iBK$_{Ca}$ has been visualized in the mitochondria (mitoBK$_{Ca}$) coincident with VDAC1 signals (Redel *et al.* 2008), and pharmacological evidence supports its role in protecting the heart from ischaemic insult as will be discussed in the following section.

Thus, it appears that there are at least three types of iBK$_{Ca}$ channels present inside the cells: (1) a pool related to the normal traffic to the plasma membrane and its regulation, (2) a pool awaiting to be translocated to the plasma membrane, and (3) another set specifically targeted to organelles. In this regard, several groups have shown the functional activity of iBK$_{Ca}$ channels in the mitochondria and nucleus (Table 1). Mechanisms that may define iBK$_{Ca}$ localization could include splice variation, β-subunit association, and/or cell-specific mechanisms.

Mitochondrial BK$_{Ca}$ channels (mitoBK$_{Ca}$)

mitoBK$_{Ca}$ channels were first identified by patch clamping of mitoplasts prepared from human glioma cells LN229 (Siemen *et al.* 1999) and later they were shown by several groups to be involved in cardioprotection against ischaemic injury by using pharmacological agents to open and block the channel. Preconditioning hearts with BK$_{Ca}$ openers like NS1619 or NS11021 reduced myocardial infarction or heart function and these beneficial effects could be antagonized by coadministration with paxilline, a commonly used BK$_{Ca}$ inhibitor (Xu *et al.* 2002; Wang *et al.* 2004; Stowe *et al.* 2006; Bentzen *et al.* 2009, 2010). Also, stimulating mitoBK$_{Ca}$ activity with β-oestradiol resulted in decreased cardiomyocyte death due to ischaemic insult (Ohya *et al.* 2005). mitoBK$_{Ca}$ has also been proposed to mediate the cardioprotective effects of the anaesthetic desflurane, the peptide adrenomedullin, and the tumour necrosis factor-α (Gao *et al.* 2005; Nishida *et al.* 2008; Redel *et al.* 2008). However, recent studies using BK$_{Ca}$ knockout (*Slo1*$^{-/-}$ or *Kcnma1*$^{-/-}$) mice have challenged the role of mitoBK$_{Ca}$ in isoflurane-mediated cardioprotection from ischaemia/reperfusion injury, and proposed a role for a large conductance K^+ channel that is activated by Na^+ (*Slo2*). Importantly, the reduction in infarct size by isoflurane preconditioning was abolished by paxilline in wild-type as well as in *Slo1*$^{-/-}$ hearts (Wojtovich *et al.* 2011) raising serious concerns about the usage of this drug as a specific blocker of BK$_{Ca}$. It would be interesting to test whether different anaesthetics use distinct cardioprotective pathways.

Mechanisms triggered by the putative opening of mitoBK$_{Ca}$ by NS1619 include regulation of reactive oxygen species (ROS) production and calcium retention capacity (CRC). In isolated mitochondria from brain and heart, mitoBK$_{Ca}$ is known to reduce ROS production on activation with NS1619 and CGS7184 (Heinen *et al.* 2007; Kulawiak *et al.* 2008), while in brain mitochondria the opening of the mitochondrial permeability transition pore (mPTP) by Ca^{2+} (indirectly measured as mitochondrial depolarization in response to Ca^{2+} pulses) is accelerated by blocking mitoBK$_{Ca}$ with iberiotoxin (Cheng *et al.* 2008). Moreover, in isolated hearts, preconditioning with NS1619 reduces ROS and mitochondrial Ca^{2+} (Stowe *et al.* 2006). Thus, it is tempting to hypothesize that reduced mitoBK$_{Ca}$ channel activity favours the opening of mPTP and *vice versa*. Consistent with this idea, the putative inhibition of mitoBK$_{Ca}$ with paxilline, induced the release of cytochrome *c*, a signature of mPTP opening and initiation of apoptosis. That inhibition of mitoBK$_{Ca}$ favours mPTP opening and apoptosis is further substantiated by the fact that the proapoptopic protein Bax can directly inhibit mitoBK$_{Ca}$ single channel activity recorded in astrocyte mitoplasts (Cheng *et al.* 2011). Conversely, the opening of mitoBK$_{Ca}$ with NS11021 improves cardiac mitochondria function by enhancing K^+ uptake without a significant change in mitochondrial membrane potential ($\Delta\Psi_m$) and improving its energetic performance (Aon *et al.* 2010).

In addition to pharmacological evidence, immunochemistry and immunogold electron microscopy have also placed BK$_{Ca}$ in the mitochondria. Western blot analysis using antibodies raised against the C-terminus of BK$_{Ca}$ channel showed a signal at ~55 kDa (Xu *et al.* 2002) or at ~125 kDa (Shi *et al.* 2007) in isolated cardiac mitochondria. Double immunostaining and confocal microscopy showed that in the cerebellum, neuronal BK$_{Ca}$ signals coincide with signals of mitochondrial proteins, OP4–1, ANT, IMM, the heat shock protein 60 (hsp60) and TIM23 (Douglas *et al.* 2006). mitoBK$_{Ca}$ signals have also been reported in rat neonatal cardiomyocytes together with VDAC1 (Redel *et al.* 2008), and data from our lab shows that it colocalizes with mitotracker in the rat embryonic heart cell line H9c2 (Fig. 2). Note that in this embryonic cell line, the majority of BK$_{Ca}$ signals are localized to mitochondria and fewer but clear signals are also observed at the cell periphery.

Table 1. iBK_{Ca} biophysical properties in mitochondria and nucleus

Organelle and method used	Cell type/ organ	Conductance and pharmacology	Recording solution $[K^+]$ (mM), $[Ca^{2+}]$ (μM)	$V_{1/2}$ or open probability (P_o)	EC_{50} for Ca^{2+}	Reference
Mitochondria Patch clamp (mitoplast)	Human glioma cell line (LN229)	295 pS ChTx-sensitive	Pipette/bath 150 K^+/150 K^+ Nominal or no Ca^{2+}/ variable Ca^{2+}	$V_{1/2} = -33 \pm 19$ mV at 8.7 μM Ca^{2+}; $V_{1/2} = 41 \pm 23$ mV at 1 μM Ca^{2+}	6.9 μM at −20 mV	Siemen *et al.* (1999)
Mitochondria Patch clamp (mitoplast)	Guinea-pig ventricular myocytes	307 pS ChTx-sensitive	150 K^+/150 K^+ 0.512 Ca^{2+}/0.512 and 40 Ca^{2+}	P_o ~0.9 at +60 mV and 0.512 μM Ca^{2+} *	N/A	Xu *et al.* (2002)
Mitochondria Patch clamp (mitoplast)	Rat ventricular myocytes	270 pS Paxilline-sensitive	140 K^+/140 K^+ 0.5 Ca^{2+}/0.5 Ca^{2+}	$P_o = 0.0087$ at +40 mV and 0.5 μM Ca^{2+}	N/A	Ohya *et al.* (2005)
Mitochondria Patch clamp (mitoplast)	Human glioma cell line (LN229 and LN405)	276 pS ChTx-sensitive	150 K^+/150 K^+ 0 Ca^{2+}/100–400 Ca^{2+}	$V_{1/2} \sim -42$ mV at 200 μM Ca^{2+} *	N/A	Gu *et al.* (2007)
Mitochondria Patch clamp (mitoplast)	Rat astrocytes	295–296 pS IbTx-sensitive; Bax-sensitive	150 K^+/150 K^+ 200 Ca^{2+}/200 Ca^{2+}	$V_{1/2} \sim -50$ mV at 200 μM Ca^{2+} *	N/A	Cheng *et al.* (2008, 2011)
Mitochondria Lipid bilayers	Rat whole brain	265 pS ChTx sensitive	(cis/trans) 50 K^+/450 K^+ 300–500 Ca^{2+}/ 300–500 Ca^{2+}	$P_o = 0.50$ at 0 Ca^{2+} $P_o = 0.77$ at $^+$70 mV and 300 μM Ca^{2+}	N/A	Skalska *et al.* (2009)
Mitochondria Lipid bilayers	Rat whole brain	211 pS IbTx-, 4-AP-sensitive; ChTx-insensitive	200 K^+/50 K^+ 'Contaminant' Ca^{2+}	$P_o = 0.9 \pm 0.01$ at +40 mV $V_{1/2} = 11 \pm 1$ mV	N/A	Fahanik-Babaei *et al.* (2011*a*)
Mitochondria Lipid bilayers	Rat whole brain	565 pS ChTx-, IbTx-, 4-AP-sensitive	200 K^+/50 K^+ 100 Ca^{2+}/100 Ca^{2+} 10 Ca^{2+}/10 Ca^{2+} 'Ca^{2+}-free'/ 'Ca^{2+}-free'	At 100 μM Ca^{2+}, $P_o = 0.9 \pm 0.05$ at −40 to +40 mV At 0 Ca^{2+}, $P_o = 0.8$ at +20 mV and $P_o = 0.07$ at −40 mV	N/A	Fahanik-Babaei *et al.* (2011*b*)
Nucleus Patch clamp	Rat pancreatic acinar cells	200 pS	Pipette/bath 148 K^+/148 K^+ 200 Ca^{2+}/0.1, 200 Ca^{2+}	$P_o = \sim 0.5$ at +40 mV* at 200 μM Ca^{2+}	N/A	Maruyama *et al.* (1995)

*Calculated from published figure. Abbreviations: $V_{1/2}$, half-activation potential or potential where an open probability of 0.5 is achieved; EC_{50}, concentration of half-maximal effect; ChTx, charybdotoxin; IbTx, iberiotoxin; 4-AP, 4-aminopyridine; N/A, not available.

Efforts to identify the molecular correlate of $mitoBK_{Ca}$ have been carried out by several groups but with limited success. A full length mRNA was cloned from mouse cardiomyocytes but the protein failed to localize to the mitochondria (Ko *et al.* 2009). Since BK_{Ca} is coded by a single gene, it is possible that a splice variant is responsible for its mitochondrial localization. In fact, in mouse cochlea where BK_{Ca} was found in the mitochondria (in addition to the cytoplasm and plasma membrane), a BK_{Ca} isoform containing four splice sequences along the C-terminus was cloned (IYF, 27 amino acids, ATRMTRMGQ, which is upstream of 50 C-terminal amino acids ending in VEDEC) (GenBank accession no. FJ872117). This cochlea clone when expressed in Chinese hamster ovary cells was observed in mitochondria with some expression at the plasma membrane (Kathiresan *et al.* 2009). However, it is not clear whether any of these splice inserts can target BK_{Ca} to the mitochondria. *In silico* analysis of the cochlea variant using MitoProt (Claros & Vincens, 1996) indicate a probability of 0.0175 for mitochondrial targeting. However, this engine searches for classical N-terminal mitochondrial signal peptides ('presequences'). It is now

known that mitochondrial targeted proteins may possess internal signals at multiple sites within the protein including the C-terminus and that inner membrane proteins may contain an internal 'presequence-like' signal. Scrutinizing the role of each of the cochlea BK$_{Ca}$ splice sequences in their ability to target mitoBK$_{Ca}$ to the mitochondria is an open topic of research.

From the functional point of view, mitoBK$_{Ca}$ conductance ranges from ∼211 pS to 565 pS depending on the biological system and experimental conditions (Table 1). Typically mitoBK$_{Ca}$ is inhibited by blockers iberiotoxin, charybdotoxin and paxilline. However, in brain mitochondrial inner membranes, mitoBK$_{Ca}$ with distinct pharmacological profiles have been detected after reconstitution into lipid bilayers, a voltage-dependent 211 pS channel that is insensitive to charybdotoxin but sensitive to iberiotoxin, and a 565 pS channel that is sensitive to both toxins. Notably, both conductances were sensitive to 10 mM 4-aminopyridine (Fahanik-Babaei *et al.* 2011*a*,*b*), a drug that does not affect cloned BK$_{Ca}$ channels (Wallner *et al.* 1995). At a high Ca^{2+} concentration (100 μM Ca^{2+}), the open probability of the channel is maintained near ∼0.9 from −40 to +40 mV; however, under Ca^{2+}-free solutions the voltage dependency of the channel becomes evident as the channel open probability changes from 0.8 at 0 mV to 0.07 at −40 mV. It is known that splice variation as well as β-subunits can confer different voltage/Ca^{2+} sensitivities to the BK$_{Ca}$ channel. For example, in the presence of the β1-subunit and Ca^{2+} near 25 μM, the open probability of the channel is around 0.9 from −50 mV onwards with a half-activation potential, $V_{1/2}$, around −100 mV (Meera *et al.* 1996). Also, β-subunits can change BK$_{Ca}$ pharmacology, for example the β4-subunit that makes BK$_{Ca}$ channels resistant to iberiotoxin (Meera *et al.* 2000). These factors could explain the variability in the single channel properties of the reported mitoBK$_{Ca}$. Whether mitoBK$_{Ca}$ isoforms with different pore properties, pharmacology and voltage sensitivities originate from splice variation and/or association with known or unknown β-subunits are relevant problems to solve.

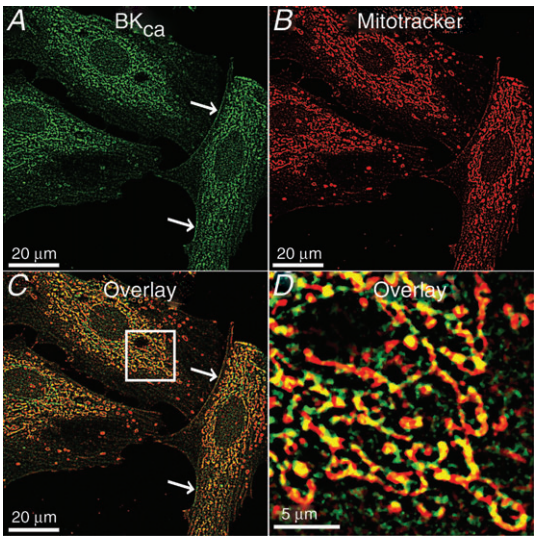

Figure 2. BK$_{Ca}$ localization in mitochondria
Cardiac H9c2 cells (rat embryonic heart cell line) were labelled with a specific BK$_{Ca}$ antibody raised against *plasma membrane* BK$_{Ca}$ channels and mitotracker. *A*, signals of BK$_{Ca}$ were readily observed inside the cell and at the cell periphery indicating plasma membrane expression (arrows). Note that in contrast to adult cardiomyocytes where BK$_{Ca}$ is absent at the plasma membrane, embryonic heart cells are known to express BK$_{Ca}$ at the plasma membrane. *B*, mitotracker labelling. *C*, overlay showing a high coincidence between BK$_{Ca}$ and mitotracker signals. *D*, square in *C* at higher magnification.

An important question is how does K^+ flux via mitoBK$_{Ca}$ affect mitochondrial function? In other words what is the physiological role of mitoBK$_{Ca}$? Attempts to answer this question have been carried out with BK$_{Ca}$ openers like NS1619 and the higher affinity analogue NS11021 using isolated cardiac mitochondria. Low doses of NS11021 (e.g. 50 nM) increase charybdotoxin-sensitive K^+ influx and swelling in the presence of permeable anions like acetate (passively diffused) and dihydrogen phosphate (carrier-mediated transport) but with very limited change (5–10 mV) in mitochondrial membrane potential ($\Delta\Psi_m$). Moreover, this K^+ influx is accompanied by a better mitochondrial respiratory control due to a decrease in state 4 respiration without a change in state 3 respiration (Aon *et al.* 2010). These properties may explain how the specific opening of mitoBK$_{Ca}$ may promote cardioprotection. Paradoxically, micromolar concentrations of NS11021 that protect the heart from ischemia and reperfusion (Bentzen *et al.* 2009) cause non-specific deleterious effects on mitochondria such as decreased respiratory control that is insensitive to charybdotoxin and a large drop in membrane potential of near 30 mV even in the absence of K^+ (Aon *et al.* 2010). Similarly, concentrations of NS1619 that cause cardioprotection (∼10 μM) (Shi *et al.* 2007) have been reported to decrease light scattering in K^+-free medium accompanied by respiration uncoupling in liver mitochondria (Bednarczyk *et al.* 2008). One possible explanation is that in whole heart experiments the effective concentration of NS1619 is actually lower due to limited diffusion of the drug to its site of action, and thus, it promotes cardioprotection instead of mitochondrial damage and heart stress. Experiments using BK$_{Ca}$ knockout animals should shed some light on these questions.

BK$_{Ca}$ channels in the nucleus

The nuclear genome and the molecular machinery required for DNA replication as well as transcription are present in the nucleus, which is sheathed by a

nuclear envelope. There are two membranes in the nuclear envelope: the inner nuclear membrane (INM) interacting with the nuclear skeleton, and the outer nuclear membrane (ONM), continuous with the ER and also studded with ribosomes. The perinuclear space between ONM and in INM is continuous with the ER lumen, so it is likely to be rich in Ca^{2+} ions. Proteins made in the ER and in the perinuclear space are transported to the lumen of ER for further trafficking in the cell. INM and ONM fuse together to form large nuclear pore complexes (NPCs) which allow bidirectional flow of large molecules. Several fundamental processes such as cell replication and differentiation, ageing, regeneration, cell cycles, and enzyme activity are governed by nuclear ionic concentrations.

K^+ channels with conductances of 55 pS and 200 pS were first recorded on the nuclear envelope from murine zygotes (Mazzanti *et al.* 1990). BK_{Ca} currents were recorded later in the ONM of rat pancreatic acinar cells (Maruyama *et al.* 1995) and observed with immunocytochemistry in chick retinal nuclei (Yamashita *et al.* 2006), the perinuclear region of isolated nuclei of brain endothelial cells (Gobeil *et al.* 2002), and in nuclei of fibroblast-like synoviocytes from patients with rheumatoid arthritis (Hu *et al.* 2012). Consistent with the electrophysiological recordings, analysis of the plasma membrane BK_{Ca} constitutive sequence by 'Nucleo', a nuclear protein localization predictor (Hawkins *et al.* 2007), yielded a score of 0.72 (1 being the perfect signal), while NucPred gave a score of 0.65 (Brameier *et al.* 2007). These *in silico* analyses suggest that BK_{Ca} might carry an intrinsic nuclear localization signal, which can target it to the nuclear membrane. However, since the same BK_{Ca} gene is present in all cells, there must be additional mechanisms targeting it to the nucleus that are cell-type specific or cell physiological-status specific.

Experiments in the nuclei of brain endothelial cells utilizing 100 nM NS1619 as BK_{Ca} opener and 100 nM iberiotoxin as a specific channel blocker indicate that nuclear iBK_{Ca} is coupled to the activity of perinuclear prostaglandin receptors (EP3) regulating nuclear Ca^{2+}, membrane potential and eNOS expression. Specifically in isolated nuclei, Ca^{2+} transients, K^+-dependent membrane potential changes and eNOS transcript expression induced by the activation of EP3 agonist M&B28767 were all abolished by iberiotoxin; while NS1619 produced Ca^{2+} transients and changes in membrane potential in 100 mM K^+ but not in 1 mM K^+ that were iberiotoxin sensitive. Further, the EP3 agonist-induced increase of eNOS expression was completely abolished by iberiotoxin mimicking the effect of Ca^{2+} chelators (Gobeil *et al.* 2002). Whether nuclear voltage-dependent R-type Ca^{2+} channels (Bkaily *et al.* 2012) are functionally coupled to iBK_{Ca}, whether iBK_{Ca} plays a role in regulating nuclear Ca^{2+} transients that occur in other cell types such as contracting chick embryonic cardiomyocytes (Bkaily *et al.* 2009), or what is the orientation and molecular nature of iBK_{Ca} in the nucleus, are open questions.

iBK_{Ca} channels in other organelles

Endoplasmic reticulum. Proteins present in the membrane of the endoplasmic reticulum (ER) are involved in protein synthesis, protein processing, protein folding, and ionic homeostasis. Enzymes working in protein synthesis and processing also require ionic homeostasis which is maintained by ion channels and transporters. Disruption in homeostasis results in accumulation of misfolded or unfolded proteins in the ER lumen. This results in ER stress which can be restored by the unfolded protein response but when this mechanism fails to remove unfolded or misfolded proteins it can result in apoptosis (Kaufman, 1999; Jing *et al.* 2012).

Similar to other proteins encoded by nuclear DNA, BK_{Ca} channels are also synthesized in the ER. Whether the iBK_{Ca} channel is active in the ER is not yet established. However, the α-subunit protein can be retained in the ER if it includes splice sequences SV1 (Zarei *et al.* 2001, 2004) or DEC (Ma *et al.* 2007). SV1 contains the ER retention motif CVLF at its first intracellular loop. This motif found in rat myometrium retains/retrieves the channel in/to the ER and also prevents BK_{Ca} surface expression. The surface expression of the protein is controlled by multiple signals in the C-terminus including an acidic cluster-like motif present in the RCK1 and RCK2 linker region DD*XX*D*XXX*I that accelerates exit from the ER (Chen *et al.* 2010) as well as six amino acids DLIFCL located near the C-terminal end (Kwon & Guggino, 2004) (Fig. 1). However, the presence of these sequences cannot override the ER-retention signal CVLF (Zarei *et al.* 2004). Interestingly, a human splice variant ($hSlo\Delta_{579–664}$) where the DD*XX*D*XXX*I motif is excluded is expressed in multiple tissues and in heterologous expression fails to form a functional ion channel at the surface and localizes the channel protein to the ER. These data strongly support the idea that one molecular mechanism defining iBK_{Ca} fate and localization to the ER (or any other organelle) is splice variation. Another mechanism contributing to BK_{Ca} localization to the ER is the presence of the β4-subunit that possesses the ER retention signal KKRKFS at its C-terminus (Shruti *et al.* 2012). Further work is needed to determine whether iBK_{Ca} localized to the ER plays a functional role.

Golgi apparatus. During protein synthesis, proteins refold and pass through the Golgi apparatus where they undergo post-translational modifications. Proteins and lipids are sorted as they exit the Golgi apparatus and are sent to their final destinations. The Golgi apparatus has

an acidic environment inside the lumen which increases from the *cis* (entry face) to the *trans* (exit face) (Anderson & Pathak, 1985); the pH in the Golgi cisternae has been estimated at 6.45 and in the *trans*-Golgi at 5.91–5.95 (Demaurex *et al.* 1998; Paroutis *et al.* 2004). The acidic environment inside the Golgi apparatus is maintained by the vacuolar-type H^+-ATPase (V-ATPase), and is essential for post-translational modifications of proteins and disruption in pH results in improperly glycosylated and unsorted proteins (Maeda & Kinoshita, 2010).

Although the V-ATPase is electrogenic in nature and would generate a positive potential inside the Golgi (Paroutis *et al.* 2004), experiments making the Golgi membrane mainly permeable to K^+ (with valinomycin) revealed that the Golgi membrane potential must be near zero under physiological conditions. This conclusion was reached after finding that valinomycin failed to change Golgi pH indicating that the Golgi membrane potential was already near the potential expected by clamping the potential with valinomycin (equilibrium potential, $E_K = -59\log[K^+]_{cytosol}/[K^+]_{Golgi} = -59\log 140/107 = -6.9$ mV) (Schapiro & Grinstein, 2000). Thus, to neutralize the membrane potential generated by the V-ATPase, H^+ or K^+ ions would need to flow out of the lumen or Cl^- ions to flow into the lumen (Paroutis *et al.* 2004). In fact, several Cl^- channels have been shown to be active in the Golgi apparatus (Nordeen *et al.* 2000; Thompson *et al.* 2002; Maeda *et al.* 2008) but so far no functional K^+ channel has been identified even though K^+ channels pass through the Golgi apparatus en route to the plasma membrane.

BK$_{Ca}$ also traffics to the plasma membrane via the *cis*- and *trans*-Golgi networks where it may undergo palmitoylation/depalmitoylation cycles with

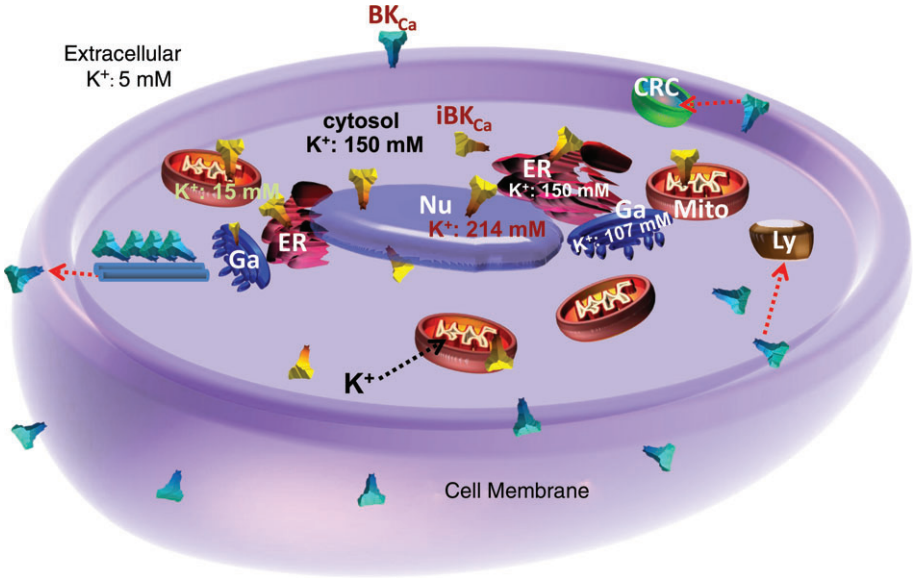

Figure 3. Intracellular organelles and potassium flow
A schematic diagram depicting the major intracellular organelles with their inner K^+ concentrations [K^+]. [K^+] inside the mitochondrion (Mito) is ∼15 mM (Zoeteweij *et al.* 1994), in the nucleus (Nu) is estimated as ∼214 mM (Nagy *et al.* 1981), and in the Golgi apparatus (Ga) is estimated as ∼107 mM (Schapiro & Grinstein, 2000). [K^+] in the ER is assumed to be similar to that in the cytoplasm, in analogy to the concentrations found in toadfish (Somlyo *et al.* 1977). Extracellular K^+ is 5 mM and cytosolic K^+ is 150 mM. BK$_{Ca}$ channels are also shown at the plasma membrane and associated with microtubules. Red arrows show translocation to clathrin reach compartments (CRC), lysosomes (Ly), and plasma membrane. Black arrow indicates the direction of K^+ ion flux upon channel opening as predicted by calculating the Gibbs free energy, ΔG, for mitochondria:
$\Delta G = \Delta G_K + \Delta G_V$,
where ΔG_K is the free energy dependent on free K^+ ions and ΔG_V is the free energy dependent on the mitochondrial potential, according to:
$\Delta G_K = -RT \ln([K^+_{cytosol}]/[K^+_{matrix}]) = -5609$ J mol^{-1}
where, R is the gas constant = 8.314 J (°K mol)$^{-1}$, and T is the absolute temperature = 293°K at 20°C, $[K^+_{cytosol}] = 150$ mM, $[K^+_{matrix}] = 15$ mM
and,
$\Delta G_V = F\Delta\Psi_m = -17367$ J mol^{-1}
where, F is the Faraday constant = 96485 J (mol V)$^{-1}$ and $\Delta\Psi_m$ is the mitochondrial membrane potential, which is typically −180 mV (Kamo *et al.* 1979).
Thus, $\Delta G = \Delta G_K + \Delta G_V = -22976$ J mol^{-1} and K^+ influx to the mitochondrial matrix is thermodynamically favoured. These equations can also be used to calculate ionic movements in other organelles.

palmitoylation favouring forward traffic to the plasma membrane of HEK293 cells (Tian *et al.* 2012). In native systems, accumulation of iBK_{Ca} in perinuclear organelles (that might include the Golgi apparatus) can be observed in myometrial cells of pregnant mouse (Eghbali *et al.* 2003) where the channel may be localized until it is needed at the plasma membrane or else playing an unknown physiological role. It is known that lowering pH can block BK_{Ca} channel unitary currents (Brelidze & Magleby, 2004) and this could indicate that if iBK_{Ca} channels are present in the Golgi, they should be most active at the *trans*-Golgi as compared to the *cis*-Golgi.

Role of iBK_{Ca} channels

In neurons, plasma membrane BK_{Ca} channels act as Ca^{2+} sensors participating in the regulation of cellular excitability and neurotransmitter release (Gribkoff *et al.* 2001). Similarly, we predict that iBK_{Ca} channels could also be working as Ca^{2+} sensors in intracellular organelles. Possibilities for iBK_{Ca} channels to get activated are either via an increase in Ca^{2+} ion concentration or by a positive shift in the membrane potential. Additionally and resembling plasma membrane channels, iBK_{Ca} could also be modulated by β-subunits (Piwonska *et al.* 2008) or G-protein-coupled receptors such as angiotensin II type 2 receptors which are present in mitochondria (Abadir *et al.* 2011) or the angiotensin II type 1 receptor present in nuclear membranes (Bkaily *et al.* 2012).

BK_{Ca} channels have a large conductance and can ideally transport $\sim 10^8$ ions per second (assuming an open probability of 1, and 25 pA at 100 mV for a 250 pS channel). The electrochemical driving force for ion movement across membranes varies with intracellular organelles; according to the calculated Gibbs free energy it is high in mitochondria (see legend of Fig. 3). The K^+ concentration in the nucleus is higher than in the cytoplasm but in all other organelles either it is equal (ER), or lower (mitochondria, Golgi) (Fig. 3). The flow of K^+ via potassium channels like iBK_{Ca} is essential to maintain this ionic homeostasis for cellular functions. Since the opening of iBK_{Ca} can result in a significant change in $[K^+]$ within organelles, in organelles with a sizeable driving force for K^+ they would be expected to be present in low abundance and/or not to fully open upon activation so organelle ionic homeostasis is not greatly disturbed during channel activation. On the other hand, if these channels were highly expressed and/or fully opened upon activation, to avoid damage they would need to be tightly modulated to bring them back to baseline or organelles would need to have alternative mechanisms to regulate their ionic homeostasis. For example in mitochondria, if BK_{Ca} were fully activated, K^+ influx could depolarize its membrane potential unless the channel open probability is tightly regulated along with coupling to other ions. In fact, a recent report indicates that the opening of BK_{Ca} does not significantly modify mitochondrial membrane potential but improves mitochondria respiratory function depending on anion usage (KH_2PO_4 *vs.* KCl) (Aon *et al.* 2010). A more complete understanding of the direct relationship between iBK_{Ca} and the modulation of membrane potential is required. In addition, iBK_{Ca} could be playing a role as a signalling molecule. It is known that BK_{Ca} interacts directly or indirectly with other proteins which can either affect the channel activity or participate in cell signalling (Lu *et al.* 2006). In line with this view, several mitochondrial, nuclear, ER, Golgi, ribosomal and peroxisomal-related proteins were also reported to be interacting with BK_{Ca} (Kathiresan *et al.* 2009). These intracellular proteins associated with iBK_{Ca} channels may be participating in organelle signalling much like those associated with its membrane counterpart.

Concluding remarks

iBK_{Ca} channels have been functionally and pharmacologically characterized by several independent groups. The variability in electrophysiological properties (Table 1) of iBK_{Ca} indicates that these channels are either splice variant isoforms and/or they are associated with modulatory subunits which can alter their biophysical properties. The presence of BK_{Ca} channels in the mitochondria is best established amongst the iBK_{Ca} channels. They are involved in physiological cellular functions such as cardioprotection. If the protection mechanism against ischaemic injury is via the opening of mitoBK_{Ca}, it could also serve as a promising pharmacological target for transplant medicine where various transplantable organs are continuously susceptible to ischaemic injury. The main challenge now is to define the molecular identity of these iBK_{Ca} channels, and their regulation and functional roles in distinct cell types.

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