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Mitochondrial ATP-sensitive potassium channel activity and hypoxic preconditioning are independent of an inwardly rectifying potassium channel subunit in *C. elegans*

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Abstract

Hypoxic preconditioning (HP) is an evolutionarily-conserved mechanism that protects an organism against stress. The mitochondrial ATP-sensitive K⁺ channel (mK_{ATP}) plays an essential role in the protective signaling, but remains molecularly undefined. Several lines of evidence suggest that mK_{ATP} may arise from an inward rectifying K⁺ channel (Kir). The genetic model organism *C. elegans* exhibits HP and displays mK_{ATP} activity. Here, we investigate the tissue expression profile of the three *C. elegans* Kir genes and demonstrate that mutant strains where the *irk* genes have been deleted either individually or in combination can be protected by HP and exhibit robust mK_{ATP} channel activity in purified mitochondria. These data suggest that the mK_{ATP} in *C. elegans* does not arise from a Kir derived channel.

Keywords

Ischemia; reperfusion; preconditioning; *C. elegans*; mK_{ATP}; Kir; *irk*

1. INTRODUCTION

Brief periods of cell stress can activate endogenous protective mechanisms that reduce injury upon subsequent exposure to stress. Ischemic preconditioning (IPC) is one such example, whereby short periods of ischemia prior to the longer ischemic insult protect against ischemia-reperfusion (IR) injury. Since its discovery [1], IPC has been described in all species tested thus far including the genetic model organism *C. elegans*, where it has been termed hypoxic preconditioning (HP) [2]. Mitochondrial K⁺ channels play an important role in protection, and the activation of the mitochondrial ATP-sensitive K⁺ channel (mK_{ATP}) is necessary and sufficient to protect against IR injury [3;4]. Since the mK_{ATP} is a critical component of IPC, the channel has been the subject of intense

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investigation and has been found in humans [5], rats [6], plants [7], amoeba [8], *T. cruzi* [9], and in *C. elegans* [10].

The molecular identity of mK_{ATP} remains elusive, but is thought to resemble the surface K_{ATP} channel, in which the pore forming subunit is a member of the inward rectifier potassium channel (Kir) family. Kir channels perform a diverse array of physiologic functions, and gene mutations have been associated with a variety of pathological conditions in humans [for review, see [11]]. The mammalian Kir gene family consists of fifteen isoforms divided into seven subfamilies (Kir1.x to Kir7.x). These subfamilies can be further classified into four functional groups, including classical, or constitutively active, G-protein gated, ATP-sensitive, and K^+ transport channels. Surface K_{ATP} channels are composed of tissue specific combinations of inward rectifying K^+ channel (Kir6.x) subunits and regulatory sulfonylurea receptor (SURx) subunits [12], and mK_{ATP} channels have been proposed to arise from some combination of these. In cardiac myocytes, the surface K_{ATP} and the mK_{ATP} resemble each other with respect to physiologic transport characteristics, but they can be distinguished by pharmacology. For example, diazoxide and 5-hydroxydecaonate (at appropriate concentrations) selectively activate and inhibit the cardiac mK_{ATP} , respectively [13]. These reagents, however, are not completely specific for the mK_{ATP} , as they can also regulate surface channel activity in other cell types, leading to the idea that the pharmacologic dissimilarity between the surface and mitochondrial channels arises from some unique combination of Kir6.x and SURx, or novel splice variants [14]. Nevertheless, despite the tools available and its critical role in the evolutionarily conserved endogenous mechanism of IPC, the mK_{ATP} has not been conclusively defined at the molecular level using pharmacologic techniques.

The use of genetic models has also failed to result in the molecular identification of the mK_{ATP} . A mouse knockout model of Kir6.2 was shown to be resistant to IPC [15], but this resistance was not related to the loss of the mK_{ATP} . This may be linked to the fact that mutations in both Kir6.2 and SUR1 have both been linked to familial persistent hyperinsulinemic hypoglycemia [16], coupled with knowledge that hyperglycemia can abrogate protection by IPC [17]. Similarly, Kir6.1 and SUR2 knockout mice both display a phenotype indicative of Prinzmetal angina [18;19], complicating an analysis of its role in IPC. However, *C. elegans* lacks a vasculature and its genome codes for only three Kir family channels, *irk-1*, *irk-2*, and *irk-3*. Hence, we hypothesized that this genetic model, which can be protected by HP [2] and exhibits a mK_{ATP} activity [10] would be a useful reductionist model to assess the role of the Kir gene family in protective signaling.

Unlike mammals, the *C. elegans* family is limited to three genes, termed *irk-1*, -2, and -3. The reduced complexity of this family compared to that in humans would be expected to facilitate dissection of Kir function in worms. However, other than the fact that they are all most closely related to Kir2.x family members, with ~50% amino acid identities, there is very little known about them. Herein, we used fluorescent transgenes to probe the cellular expression pattern and protein localization of all three Kir genes (*irk-1*, -2, and -3) in *C. elegans*. Using worm genetic knockouts we determined if these genes were involved in HP or contributed to mK_{ATP} activity in isolated mitochondria. Our results suggest that the worm IRK channels have a limited distribution, which is inconsistent with a conserved function in mitochondria, and do not contribute to either HP or mK_{ATP} activity.

2. MATERIALS AND METHODS

2.1 *C. elegans*

Worm strains were routinely propagated at 20°C on nematode growth media agar plates containing 5 µg/ml cholesterol and seeded with OP50 bacteria. Strains used in this study

include Bristol-N2; fluorescent protein fusion strains (all in a *pha-1(e2123ts)III*, *him-5(e1490)V* genetic background) KWN73 rnyEx040 [pKT48 (*Pirk-1::IRK-1::GFP*); pCL1 (*pha-1+*)], KWN83 rnyEx043 [pKT54 (*Pirk-3::GFP*); pCL1 (*pha-1+*)], KWN88 rnyEx045 [pKT49 (*Pirk-2::IRK-2::GFP*); pCL1 (*pha-1+*)]; and *irk* gene deletion strains MT15934 *irk-1(n4895)X*, MT15935 *irk-2(n4896)X*, MT17369 *irk-3(n5049)X*, MT17360 *irk-1(n4895)X*, *irk-2(n4896)X*, *irk-3(n5049)X* (these mutants were generously provided by N. Ringstaad and R. Horvitz). The triple deletion was obtained by a conventional mating strategy with PCR genotyping used to confirm the presence of the mutant alleles in the final strain.

2.2 Fluorescent Imaging

Transgenic *C. elegans* strains expressing the fluorescent *irk* gene fusions were placed onto 2% M9 agarose pads under anesthesia (1 mg/ml tetramisole) and examined using an Olympus FV1000 confocal microscope (available as part of the University of Rochester Confocal Core) under appropriate illumination.

2.3 IR killing and HP of *C. elegans*

Briefly, *C. elegans* were chronologically-synchronized and adult hermaphrodites were collected, washed and allowed to lay embryos for a period of two hours. The resulting adult progeny were subject to the following protocols as previously described [2]. **Simulated ischemia-reperfusion (IR) killing:** *C. elegans* in M9 were incubated at 26°C for 20 hrs in an anoxic chamber (5%/95% H₂/N₂). Following the ischemic insult, *C. elegans* were returned to normoxia at 20°C for 24 hrs. Animals were then scored. Animals which lack movement (spontaneous or touch-evoked) were scored as dead. **Hypoxic preconditioning (HP):** *C. elegans* were incubated for 4 hrs in an anoxic chamber at 26°C. Following the stimulus, *C. elegans* were moved to normoxia at 20°C for 20 hours to allow for recovery. The preconditioned *C. elegans* were then subjected to IR killing as described above. For all experiments, seven replicates of at least thirty *C. elegans* were observed under paired IR and HP protocols for each of the mutant strains.

2.4 Isolation of mitochondria, BTC-AM loading, and mK_{ATP} thallium flux assay (Tl⁺-flux)

Mitochondria were isolated from *C. elegans* as previously described [10]. The mitochondrial suspension was placed in a chilled stirring cuvette and incubated in the dark with 20 μM BTC-AM (Invitrogen) and 0.05% Pluronic F-127 for 10 min at room temperature. Mitochondria were washed to remove excess BTC-AM by centrifugation at 7000 × g for 10 min. The pellet was resuspended in MIM (220 mM mannitol, 70 mM sucrose, 5 mM MOPS, 2 mM EGTA, 0.4% BSA, pH 7.4, filtered), stored on ice in the dark and used within 1.5 hours of isolation. Protein was determined by the Folin-phenol method [7].

mK_{ATP} activity was determined by monitoring the uptake of Tl⁺, a surrogate for K⁺, into mitochondria using a Varian Cary Eclipse spectrofluorometer as previously described [20]. Briefly, ~0.8 mg/ml BTC-AM-loaded mitochondria were added to a rapidly stirred cuvette containing 2 ml of Tl⁺ assay buffer (2 mM pyruvate, 2 mM malate, 195 mM mannitol, 10 mM HEPES, 2 mM MgCl₂, 2 mM Na₂HPO₄, and 1 μg/ml oligomycin, pH 7.2 at 25°C). Compounds were present in the buffer and incubated with mitochondria for 30 seconds. Tl₂SO₄ (2 mM) was added via a syringe port and the change in fluorescence was monitored (λ_{ex} 488 nm, λ_{em} 525 nm) and normalized to an initial baseline recording 10 seconds.

2.5 Reagents

Chemicals were of the highest grade available from Sigma (St. Louis, MO) unless otherwise specified. BTC-AM was from Invitrogen (Carlsbad, CA). Atpenin A5 was from Axxora LLC (San Diego, CA),

2.6 Statistics

In isolated mitochondrial studies, statistical significance ($P < 0.05$) between multiple groups was determined using analysis of variance (ANOVA). In whole worm studies, significance ($P < 0.05$) was determined using a paired Student's *t*-test. Data presented are mean $\pm$ SEM.

3. RESULTS

3.1 Expression profiles of *irk* genes

C. elegans hermaphrodites are comprised of ~1000 somatic cells that share many basic cell biologic processes with mammals. In order to define the cells and cell types that express the *irk* genes, each of the three *irk* promoters was amplified using genomic PCR and fused via PCR sewing to the coding region of GFP. These PCR products were used to create transgenic nematode strains where the fluorescent cells demarked the limits of *irk* gene expression (transcriptional reporters *Pirk-x::GFP*). As a complementary approach, the entire *irk* coding sequence was amplified together with its cognate promoter and fused in-frame to GFP (translational reporters *Pirk-x::IRK-x::GFP*). Translational reporters give rise to fluorescent IRK fusion proteins that can be used to determine intracellular protein localization. It should be noted when examining worms expressing these constructs that promoter-driven transgenes may not completely recapitulate endogenous expression patterns, either due to mosaicism (where individual worms may not exhibit the full repertoire of cells due to loss of the transgene during mitosis) or because sequences in the genomic region such as the 3' UTR contribute to proper expression.

Figure 1 shows representative images of transgenic strains expressing fluorescent reporters for each of the *irk* genes. For the *irk-1* and *irk-2* genes, their respective transcriptional and translational fusions were expressed in a similar repertoire of cells (data not shown), and hence only the translational fusions are shown. The *irk-1* promoter drove GFP expression in multiple neurons, including the hermaphrodite specific neuron (HSN), sensory phasmid neurons in the tail, and unidentified neurons in both the head and tail of male worms (Figure 1A–D). Intestinal and gonadal cells were labeled as well (Figure 1E–F). As would be predicted for all of the IRK proteins based upon homology with the mammalian Kir channels, clear plasma-membrane targeting of the GFP-tagged IRK-1 protein can be observed in the intestine (Figure 1C and 1E). This does not preclude dual targeting of the protein to the mitochondria, however, and it should be noted that enforced expression of fluorescent fusion proteins in worms can lead to targeting artifacts. However, the *irk-1* cDNA expressed in *Xenopus* oocytes gave rise to a plasma membrane K^+ conductance (N. Ringstad, personal communication), suggesting that IRK-1 is a bona fide plasma membrane channel. The fusion protein was likely active, as well, since strains expressing it were viable but were not healthy, and exhibited signs of cellular degeneration over time. For example, the neuronal processes in adults assumed a tortuous morphology and the fusion proteins appeared to aggregate over time (Figure 1A). This can occur due to overexpression or misregulation of a “leak” conductance type channel that is normally only active under select conditions.

As for IRK-1, IRK-2 is also expected to be targeted to the plasma membrane. The most notable cells labeled by *irk-2* promoter expression were neurons of the anterior ganglia, the pharyngeal intestinal valve cell, and the posterior intestine, where again clear basolateral

plasma membrane targeting can be observed (Figure 1G–L). In adults, the spermatheca and the uterus were prominently labeled (Figure 1K), and the IRK-2 fusion protein resided in the plasma membrane.

The *irk-3* translational fusion was not tolerated by the worms, even at low doses. Hence, a transcriptional fusion was used to assess the extent of *irk-3* gene expression. *Irk-3* promoter activity was limited to neurons, but it was broadly expressed throughout multiple neuronal subtypes, including sensory neurons and the HSN (Figure 1M–O).

It is interesting that *irk* gene expression was notably absent in muscle and hypodermal cells. These cell types are amongst the largest in *C. elegans* and would be expected to contribute greatly to their total mitochondrial content. A channel that is localized to the mitochondria, even in part, and contributes to mitochondrial protection from insult might be predicted to be expressed more ubiquitously than observed here for the *irk* genes.

3.2 Hypoxic preconditioning in *C. elegans* is independent of *irk* genes

Since HP is widely conserved across species and is known to protect *C. elegans* from hypoxic death [2], and worms mitochondria exhibit robust mK_{ATP} channel activity [10], we sought to determine whether the *C. elegans* mK_{ATP} channel is comprised of a Kir family gene product. Mammals contain fifteen Kir genes, while *C. elegans* only contains three genes in the Kir family, termed *irk-1*, *irk-2*, and *irk-3*. As is often the case with large gene families, it is difficult to identify mammal-worm orthologs, and the worm genes are more closely related to one another than to any of the mammalian isoforms. The mammalian gene product most closely related to the worm *irk* family is Kir2.1. However, it should be noted that K_{ATP} channels open spontaneously in the absence of ATP and can be broadly classified with the classical Kir2.x channels which have constitutive activity.

C. elegans loss-of-function mutants in the *irk-1*, *irk-2*, and *irk-3* genes are viable, and due to the absence of tissues that are known to depend on K_{ATP} function in mammals (e.g. pancreatic beta-cells) in the worm, we hypothesized that they would be less likely to present confounding issues. First, we sought to determine if the *irk* gene products were required for HP. The worm HP model is a model of delayed preconditioning where a short 4 hr hypoxic exposure, followed by 20 hrs of normoxic recovery, induces resistance to a subsequent 24 hrs hypoxic killing. As shown in Figure 2, HP significantly protected the wild-type (WT) strain from hypoxic stress, as previously demonstrated [2;21]. Unexpectedly, all of the *irk* deletion strains were also protected by HP to the same extent as WT, as was a triple deletion strain containing combined *irk-1*, *irk-2*, and *irk-3* mutant alleles (Figure 2). However, the triple deletion strain exhibited somewhat less variability in its basal sensitivity to hypoxia than the WT and, though statistically insignificant, appeared slightly more resistant to hypoxia on average (WT, 60% death; triple, 50% death; p-value 0.30). In conclusion, these results suggest that HP in *C. elegans* is independent of a Kir family K⁺ channel.

3.3 mK_{ATP} channel activity in *C. elegans* is independent of *irk* genes

We next sought to determine whether *C. elegans* mK_{ATP} activity was affected by the loss of *irk* gene function in the deletion mutants. The mK_{ATP} contributes to K⁺ transport across the mitochondrial inner membrane, and channel activity can be monitored by fluorescent measurements of TI⁺ fluxes (as a surrogate for K⁺) in isolated mitochondria using the dye BTC-AM [20;21]. Using this technique, we measured changes in mitochondrial TI⁺ uptake in response to a conserved repertoire of activators and inhibitors that together define a pharmacologic signature for mK_{ATP} activity. We then compared this signature in WT worms to that in the *irk-1/-2/-3* triple deletion mutant to assess the contribution of worm *irk* genes to mK_{ATP} activity.

In WT worms we observed mitochondrial Ti^+ flux that was inhibited by ATP and activated by both diazoxide and atpenin A5 (Figure 3). This activation by channel openers could be inhibited by mK_{ATP} blockers 5-hydroxydecanoate and glyburide (Figure 3), consistent with mK_{ATP} activity in isolated *C. elegans* as well as mammalian mitochondria [10]. Mitochondria from the triple *irk* deletion strain exhibited a nearly identical profile, providing further evidence that mK_{ATP} is not derived from a Kir protein in *C. elegans*.

4. DISCUSSION

The mK_{ATP} is central to IPC-mediated protection yet its molecular identity has remained elusive. The simplest hypothesis put forth thus far has been that the protein composition of the mK_{ATP} resembles that of the defined surface K_{ATP} , which is comprised of an octamer of four Kir channel subunits and four SUR accessory subunits. However, our results demonstrate that neither HP nor mK_{ATP} requires Kir gene function in the nematode *C. elegans*.

One interesting outcome of the current data is the possibility that Kir genes are also not involved in the mK_{ATP} in mammals. Significant effort has been directed toward assessing a role for putative K_{ATP} components (e.g. Kir6.2, Kir6.1 and SUR) in mammalian mitochondria, with ambiguous results. These efforts can be broadly categorized into three areas. The first of these relies upon antibody detection, the second on genetic elimination of candidates, and the third on pharmacologic manipulation of channel activity.

Approaches based primarily on antibody labeling concluded that either Kir6.2 [5], Kir6.1 [22–24], both [25–27] or neither [28] were present in mitochondria, perhaps due to variability in reagent quality. In this regard, two commercially available anti-Kir6.1 antibodies were shown to recognize off-target mitochondrial proteins, NADH-dehydrogenase flavoprotein 1 and isocitrate dehydrogenase [29]. Using custom antibodies, a novel splice variant of SUR2 has been identified in cardiac mitochondria [14], but its relevance to mK_{ATP} is as of yet unclear.

To attempt to circumvent this type of confusion, genetic loss-of-function mouse models have been developed for Kir6.1 [19], Kir6.2 [16], SUR1 [30], and SUR2 [31]. However, these models exhibited either incomplete loss of gene activity [32], or confounding effects (e.g. elevated blood glucose) that prevented unambiguous interpretation of their ability to be effectively protected by IPC [16–19]. Despite this, a technique utilizing flavoprotein fluorescence has been employed as an indirect measurement of mK_{ATP} activity in genetic deletion or dominant negative gene transfer models, and neither Kir6.1 nor Kir6.2 has been found to play an important role in mK_{ATP} opening-induced flavoprotein fluorescence [15;33]. This result is in general agreement with our finding that none of the Kir genes plays a role in mK_{ATP} activity in worms. Furthermore, the mK_{ATP} has been described in plants recently, where the single Kir gene was hypothesized to be unlikely to contribute to the formation of the mK_{ATP} [34]. Clearly, genetic deletion of all 15 Kir isoforms in mammals is not practical, but these results do suggest that the search for the mK_{ATP} should explore other non-Kir candidates.

Pharmacologic approaches to studying mK_{ATP} have been similarly ineffective at channel identification. In general, compounds which open the surface K_{ATP} activate the mK_{ATP} and cause cardioprotection. Likewise, inhibitors of the surface K_{ATP} block the mK_{ATP} . This cross-reactivity suggests some degree of conservation between these two channels, but makes it difficult to study the role of either one in isolation. This fact along with other shortcomings has caused some to question whether a mK_{ATP} channel contributes to cardioprotection or exists at all [35]. However, there are a few pharmacologic tools that have

used to dissect mK_{ATP} from the surface K_{ATP} . At low concentrations in cardiac myocytes, diazoxide and 5-HD are specific mK_{ATP} modulators and do not regulate the surface channel [13]. However, even in myocytes at higher concentrations they have additional effects that might be predicted to impact IPC independent of their effect on mK_{ATP} [36;37]. These alternate targets may actually provide some insight into the molecular nature of the mK_{ATP} .

For example, the specific activator of the mK_{ATP} , diazoxide, also inhibits complex II of the mitochondrial respiratory chain [36]. This characteristic is not unique to diazoxide, as the same result was observed for malonate [38] and atpenin A5 [39] amongst other complex II inhibitors [40]. Yet another study demonstrated that a purified mitochondrial inner membrane fraction displayed K^+ channel activity that was sensitive to mK_{ATP} activators and blockers but also responded to complex II inhibitors [28]. Hence, a moonlighting role for complex II in mitochondrial K^+ transport has been hypothesized.

While the data presented herein demonstrate that a Kir isoform is not involved in the composition of the mK_{ATP} , they do not rule out the participation of a SUR subunit. In this regard, mK_{ATP} activity can be inhibited with the sulfonylurea glyburide (glybenclamide) even in the *irk* triple mutant (Figure 3). It is possible that a SUR might associate with an alternate K^+ -transporting subunit to give rise to mK_{ATP} activity. It is also possible, as discussed for diazoxide, that glyburide may have an alternative target [41;42]. In this respect, glyburide has been shown to interact with mitochondrial ADP/ATP carriers [43]. Further work is undoubtedly necessary to elucidate the role of a SUR subunit in the composition of the mK_{ATP} .

Another interesting candidate arises from recent work examining the regulation of mK_{ATP} by redox status [40]. While a general trend of channel inhibition was observed with reducing agents of increasing redox potential, it was observed NADPH did not conform to this trend, i.e., despite identical redox potentials, NADPH was far more inhibitory to channel function than NADH. This suggests that NADPH is able to inhibit mK_{ATP} via a non-redox mechanism. Notably, the beta subunit of the “Shaker” family Kv channels has been shown to contain an NADPH binding site, coupled to an aldo-ketoreductase enzymatic activity [44;45]. In addition, single channel patch-clamp experiments from plant mitochondria are consistent with a Shaker like conductance, though the electro-physiologic characteristics of the single plant Kir gene product are unknown [34]. This raises the intriguing possibility that an NADPH binding Kv-beta subunit may be involved in mK_{ATP} assembly and/or regulation. Similarly intriguing perhaps is the observation that Kv1.3 forms a mitochondrial channel in lymphocytes that regulates apoptosis [46;47].

5. CONCLUSIONS

The quest for the molecular identity of mK_{ATP} channel remains. Antibody based approaches, pharmacologic regulatory strategies and mammalian genetic models have led to ambiguous conclusions and suffer from confounding effects. Furthermore, compensatory mechanisms involving other Kir subunits may rescue the mK_{ATP} activity in knockout animals. The data presented herein demonstrate effective HP and normal mK_{ATP} activity in *C. elegans* that do not have any of the three inwardly rectifying K^+ channel genes. Furthermore, the use of a strain where all the Kir family genes in *C. elegans* have been knocked out together rules out the possibility of compensatory mechanisms involving another Kir isoform. These results suggest that the evolutionarily conserved phenomena of the mK_{ATP} and IPC may not center on a Kir component and should drive investigation of alternate targets.

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ABBREVIATIONS

BTC-AM	benzothiazole coumarin acetyoxymethyl ester
IPC	ischemic preconditioning
mK_{ATP}	mitochondrial ATP-sensitive K ⁺ channel
WT	wild-type
IRK (Kir)	inwardly rectifying potassium channel subunit

References

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986; 74:1124–1136. [PubMed: 3769170]
2. Dasgupta N, Patel AM, Scott BA, Crowder CM. Hypoxic preconditioning requires the apoptosis protein CED-4 in *C. elegans*. *Curr Biol*. 2007; 17:1954–1959. [PubMed: 17980592]
3. Facundo HT, Fornazari M, Kowaltowski AJ. Tissue protection mediated by mitochondrial K⁺ channels. *Biochim Biophys Acta*. 2006; 1762:202–212. [PubMed: 16026967]
4. Garlid KD, Dos SP, Xie ZJ, Costa AD, Paucek P. Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K(+) channel in cardiac function and cardioprotection. *Biochim Biophys Acta*. 2003; 1606:1–21. [PubMed: 14507424]
5. Jiang MT, Ljubkovic M, Nakae Y, Shi Y, Kwok WM, Stowe DF, Bosnjak ZJ. Characterization of human cardiac mitochondrial ATP-sensitive potassium channel and its regulation by phorbol ester in vitro. *Am J Physiol Heart Circ Physiol*. 2006; 290:H1770–H1776. [PubMed: 16361367]
6. Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K⁺ channel in the mitochondrial inner membrane. *Nature*. 1991; 352:244–247. [PubMed: 1857420]
7. Pastore D, Stoppelli MC, Di FN, Passarella S. The existence of the K(+) channel in plant mitochondria. *J Biol Chem*. 1999; 274:26683–26690. [PubMed: 10480870]
8. Kicinska A, Swida A, Bednarczyk P, Koszela-Piotrowska I, Choma K, Dolowy K, Szewczyk A, Jarmuszkiewicz W. ATP-sensitive potassium channel in mitochondria of the eukaryotic microorganism *Acanthamoeba castellanii*. *J Biol Chem*. 2007; 282:17433–17441. [PubMed: 17430885]
9. Costa ADT, Krieger MA. Evidence for an ATP-sensitive K⁺ channel in mitoplasts isolated from *Trypanosoma cruzi* and *Crithidia fasciculata*. *International Journal for Parasitology*. 2009; 39:955–961. [PubMed: 19504755]
10. Wojtovich AP, Burwell LS, Sherman TA, Nehrke KW, Brookes PS. The *C. elegans* mitochondrial K⁺(ATP) channel: a potential target for preconditioning. *Biochem Biophys Res Commun*. 2008; 376:625–628. [PubMed: 18809388]
11. Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y. Inwardly rectifying potassium channels: their structure, function, and physiological roles. *Physiol Rev*. 2010; 90:291–366. [PubMed: 20086079]
12. Nichols CG. KATP channels as molecular sensors of cellular metabolism. *Nature*. 2006; 440:470–476. [PubMed: 16554807]
13. Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, Grover GJ. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K⁺ channels. Possible mechanism of cardioprotection. *Circ Res*. 1997; 81:1072–1082. [PubMed: 9400389]

14. Ye B, Kroboth S, Pu JL, Sims J, Aggarwal N, McNally E, Makielski J, Shi NQ. Molecular Identification and Functional Characterization of a Mitochondrial Sulfonylurea Receptor 2 Splice Variant Generated by Intraexonic Splicing. *Circ Res*. 2009
15. Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M, Seino S, Marban E, Nakaya H. Role of sarcolemmal K(ATP) channels in cardioprotection against ischemia/reperfusion injury in mice. *J Clin Invest*. 2002; 109:509–516. [PubMed: 11854323]
16. Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gono T, Iwanaga T, Miyazaki J, Seino S. Defective insulin secretion and enhanced insulin action in KATP channel-deficient mice. *Proc Natl Acad Sci U S A*. 1998; 95:10402–10406. [PubMed: 9724715]
17. Kersten JR, Toller WG, Gross ER, Pagel PS, Warltier DC. Diabetes abolishes ischemic preconditioning: role of glucose, insulin, and osmolality. *Am J Physiol Heart Circ Physiol*. 2000; 278:H1218–H1224. [PubMed: 10749717]
18. Chutkow WA, Pu J, Wheeler MT, Wada T, Makielski JC, Burant CF, McNally EM. Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K(ATP) channels. *J Clin Invest*. 2002; 110:203–208. [PubMed: 12122112]
19. Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K, Koseki H, Iwanaga T, Nakaya H, Seino S. Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat Med*. 2002; 8:466–472. [PubMed: 11984590]
20. Wojtovich AP, Williams DM, Karcz MK, Lopes CM, Gray DA, Nehrke KW, Brookes PS. A Novel Mitochondrial KATP Channel Assay. *Circ Res*. 2010; 106:1190–1196. [PubMed: 20185796]
21. Wojtovich AP, Sherman TA, Nadtochiy SM, Urciuoli WR, Brookes PS, Nehrke K. SLO-2 Is Cytoprotective and Contributes to Mitochondrial Potassium Transport. *PLoS One*. 2011; 6:e28287. [PubMed: 22145034]
22. Suzuki M, Kotake K, Fujikura K, Inagaki N, Suzuki T, Gono T, Seino S, Takata K. Kir6.1: a possible subunit of ATP-sensitive K⁺ channels in mitochondria. *Biochem Biophys Res Commun*. 1997; 241:693–697. [PubMed: 9434770]
23. Zhou M, Tanaka O, Sekiguchi M, Sakabe K, Anzai M, Izumida I, Inoue T, Kawahara K, Abe H. Localization of the ATP-sensitive potassium channel subunit (Kir6.1/uK(ATP)-1) in rat brain. *Brain Res Mol Brain Res*. 1999; 74:15–25. [PubMed: 10640672]
24. Zhou M, He HJ, Suzuki R, Tanaka O, Sekiguchi M, Yasuoka Y, Kawahara K, Itoh H, Abe H. Expression of ATP sensitive K⁺ channel subunit Kir6.1 in rat kidney. *Eur J Histochem*. 2007; 51:43–51. [PubMed: 17548268]
25. Singh H, Hudman D, Lawrence CL, Rainbow RD, Lodwick D, Norman RI. Distribution of Kir6.0 and SUR2 ATP-sensitive potassium channel subunits in isolated ventricular myocytes. *J Mol Cell Cardiol*. 2003; 35:445–459. [PubMed: 12738227]
26. Lacza Z, Snipes JA, Miller AW, Szabo C, Grover G, Busija DW. Heart mitochondria contain functional ATP-dependent K⁺ channels. *J Mol Cell Cardiol*. 2003; 35:1339–1347. [PubMed: 14596790]
27. Lacza Z, Snipes JA, Kis B, Szabo C, Grover G, Busija DW. Investigation of the subunit composition and the pharmacology of the mitochondrial ATP-dependent K⁺ channel in the brain. *Brain Res*. 2003; 994:27–36. [PubMed: 14642445]
28. Ardehali H, Chen Z, Ko Y, Mejia-Alvarez R, Marban E. Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K⁺ channel activity. *Proc Natl Acad Sci U S A*. 2004; 101:11880–11885. [PubMed: 15284438]
29. Foster DB, Rucker JJ, Marban E. Is Kir6.1 a subunit of mitoK(ATP)? *Biochem. Biophys Res Commun*. 2008; 366:649–656.
30. Shiota C, Larsson O, Shelton KD, Shiota M, Efanov AM, Hoy M, Lindner J, Koopitwut S, Juntti-Berggren L, Gromada J, Berggren PO, Magnuson MA. Sulfonylurea receptor type 1 knock-out mice have intact feeding-stimulated insulin secretion despite marked impairment in their response to glucose. *J Biol Chem*. 2002; 277:37176–37183. [PubMed: 12149271]
31. Chutkow WA, Samuel V, Hansen PA, Pu J, Valdivia CR, Makielski JC, Burant CF. Disruption of Sur2-containing K(ATP) channels enhances insulin-stimulated glucose uptake in skeletal muscle. *Proc Natl Acad Sci U S A*. 2001; 98:11760–11764. [PubMed: 11562480]

32. Pu JL, Ye B, Kroboth SL, McNally EM, Makielski JC, Shi NQ. Cardiac sulfonylurea receptor short form-based channels confer a glibenclamide-insensitive KATP activity. *J Mol Cell Cardiol.* 2008; 44:188–200. [PubMed: 18001767]
33. Seharaseyon J, Ohler A, Sasaki N, Fraser H, Sato T, Johns DC, O'Rourke B, Marban E. Molecular composition of mitochondrial ATP-sensitive potassium channels probed by viral Kir gene transfer. *J Mol Cell Cardiol.* 2000; 32:1923–1930. [PubMed: 11185581]
34. De MU, Checchetto V, Zanetti M, Teardo E, Soccio M, Formentin E, Giacometti GM, Pastore D, Zoratti M, Szabo I. ATP-sensitive cation-channel in wheat (*Triticum durum* Desf): identification and characterization of a plant mitochondrial channel by patch-clamp. *Cell Physiol Biochem.* 2010; 26:975–982. [PubMed: 21220928]
35. Das M, Parker JE, Halestrap AP. Matrix volume measurements challenge the existence of diazoxide/glibenclamide-sensitive KATP channels in rat mitochondria. *J Physiol.* 2003; 547:893–902. [PubMed: 12562892]
36. Schafer G, Wegener C, Portenhauser R, Bojanovski D. Diazoxide, an inhibitor of succinate oxidation. *Biochem Pharmacol.* 1969; 18:2678–2681. [PubMed: 4327387]
37. Hanley PJ, Gopalan KV, Lareau RA, Srivastava DK, von MM, Daut J. Beta-oxidation of 5-hydroxydecanoate, a putative blocker of mitochondrial ATP-sensitive potassium channels. *J Physiol.* 2003; 547:387–393. [PubMed: 12562916]
38. Wojtovich AP, Brookes PS. The endogenous mitochondrial complex II inhibitor malonate regulates mitochondrial ATP-sensitive potassium channels: Implications for ischemic preconditioning. *Biochim Biophys Acta.* 2008; 1777:882–889. [PubMed: 18433712]
39. Wojtovich AP, Brookes PS. The complex II inhibitor atpenin A5 protects against cardiac ischemia-reperfusion injury via activation of mitochondrial KATP channels. *Basic Res Cardiol.* 2009; 104:121–129. [PubMed: 19242645]
40. Queliconi BB, Wojtovich AP, Nadtochiy SM, Kowaltowski AJ, Brookes PS. Redox regulation of the mitochondrial K(ATP) channel in cardioprotection. *Biochim Biophys Acta.* 2011; 1813:1309–1315. [PubMed: 21094666]
41. Jaburek M, Yarov-Yarovoy V, Paucek P, Garlid KD. State-dependent inhibition of the mitochondrial KATP channel by glyburide and 5-hydroxydecanoate. *J Biol Chem.* 1998; 273:13578–13582. [PubMed: 9593694]
42. Szewczyk A, Pikula S, Wojcik G, Nalecz MJ. Glibenclamide inhibits mitochondrial K⁺ and Na⁺ uniports induced by magnesium depletion. *Int J Biochem Cell Biol.* 1996; 28:863–871. [PubMed: 8811835]
43. Ziemys A, Toleikis A, Kopustinskiene DM. Molecular modelling of K(ATP) channel blockers-ADP/ATP carrier interactions. *Syst Biol (Stevenage).* 2006; 153:390–393. [PubMed: 16986324]
44. Pan Y, Weng J, Cao Y, Bhosle RC, Zhou M. Functional coupling between the Kv1.1 channel and aldo-ketoreductase Kvbeta1. *J Biol Chem.* 2008; 283:8634–8642. [PubMed: 18222921]
45. Pan Y, Weng J, Levin EJ, Zhou M. Oxidation of NADPH on Kvbeta1 inhibits ball-and-chain type inactivation by restraining the chain. *Proc Natl Acad Sci U S A.* 2011; 108:5885–5890. [PubMed: 21436029]
46. Gulbins E, Sassi N, Grassme H, Zoratti M, Szabo I. Role of Kv1.3 mitochondrial potassium channel in apoptotic signalling in lymphocytes. *Biochim Biophys Acta.* 2010; 1797:1251–1259. [PubMed: 20114030]
47. Szabo I, Bock J, Grassme H, Soddemann M, Wilker B, Lang F, Zoratti M, Gulbins E. Mitochondrial potassium channel Kv1.3 mediates Bax-induced apoptosis in lymphocytes. *Proc Natl Acad Sci U S A.* 2008; 105:14861–14866. [PubMed: 18818304]

HIGHLIGHTS

- A mitochondrial K_{ATP} channel (mK_{ATP}) is central to ischemic preconditioning (IPC).
- A Kir family member is thought to form the mK_{ATP} .
- *C. elegans* benefit from IPC and display mK_{ATP} activity.
- Kir genes in worms are not required for IPC or mK_{ATP} .

Fig. 1.

Expression analysis of the *irk* genes in *C. elegans*. The promoter and genomic ORFs of *irk-1* (A–F) and *irk-2* (G–L) and the promoter of *irk-3* (M–P) were amplified via PCR, fused to the ORF of GFP, and micro-injected to create transgenic strains. Representative fluorescent confocal (A, C, E, G, I, K, M, and O) and Normarski (B, D, F, H, J, L, N, and P) images are shown indicating the cells where the promoter is active. The *irk-1* and *irk-2* translational fusions additionally demonstrate plasma membrane localization of the respective fusion proteins. The worms in panels C and D are male (♂). Abbreviations: ag, anterior ganglia; AF, intestinal autofluorescence; bwm, body wall muscle; gon, gonad; gsc, gonadal sheath cell; HSN, hermaphrodite specific neurons; int, intestine; mec, mechanosensory neurons; pg, posterior ganglia; phs, phasmid neurons; piv, pharyngeal intestinal valve cells; spt, spermatheca; ut, uterus.

Fig. 2. HP in *C. elegans* is independent of the Kir family gene products *irk-1*, -2, and -3. (A) *C. elegans* WT control (N2-Bristol), and mutant deletion strains containing either the *irk-1*(n4895)X, *irk-2*(n4896)X, or *irk-3*(n5049)X alleles or all three mutant alleles in combination (triple) were subjected to simulated ischemia reperfusion (IR; white squares) or hypoxic preconditioning plus IR (HP+IR, gray squares) as detailed in the methods. Viability is expressed as percent of dead worms. To minimize handling artifacts, paired trials were performed on the same day using worms of identical age, and the IR and HP+IR values for each paired trial are linked together in the plot. (B) In order to compare different mutants, the degree of protection afforded by HP (equivalent to the IR minus the HP+IR for each paired trial) was averaged across the seven trials for each genotype. Means $\pm$ SEM, N=7, (N = independent trials performed on separate days, with each trial consisting of the average value obtained from 3 plates of worms, with >50 worms per plate), *p<0.05 vs. IR.

Fig. 3.

C. elegans mK_{ATP} channel activity is independent of the Kir family gene products *irk-1*, -2, and -3. *C. elegans* mK_{ATP} channel activity in mitochondrial purified from N2-Bristol control worms (WT; white bars) or the *irk-1(n4895);irk-2(n4896);irk-3(n5049)* triple mutant (triple; gray bars). The baseline Δ fluorescence (Ctrl, set to 100%) was 15.3 ± 3.0 and 15.8 ± 2.7 arbitrary units for the WT and triple mutant, respectively. Pharmacologic agents were present as listed below the x-axis. Abbreviations: AA5, Atpenin A5; DZX, diazoxide; Gly, Glyburide; 5HD, 5-hydroxydecanoate. Data are means $\pm$ SEM, $N > 4$ (N =independent mitochondria preparations). # $p < 0.05$ vs. Ctrl., * $p < 0.05$ vs. ATP, † $p < 0.05$ vs. ATP+DZX or ATP+AA5.