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A New pROM King for the mitoK_{ATP} Dance: ROMK Takes the Lead

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Ischemic heart disease remains the leading cause of death in the developed world, and mechanisms to reduce cardiac ischemic damage are being actively investigated. A potent protective mechanism for the heart is ischemic preconditioning (IPC), a phenomenon in which brief ischemic episodes protect the heart from tissue damage and cell death resulting from subsequent periods of ischemia and reperfusion¹. The precise molecular mechanisms at play during IPC are not known, but the opening of a mitochondrial ATP-sensitive potassium channel, mitoK_{ATP}, is believed to be necessary for IPC-induced activation of several pro-survival pathways and processes².

Although the discovery of a putative mitoK_{ATP} occurred over twenty years ago³, progress on identifying its molecular composition has been limited. The mitoK_{ATP} was determined to be both functionally and molecularly distinct from sarcolemmal K_{ATP} channels, which have a relatively minimal role in IPC protection and are insensitive to several drugs that affect mitoK_{ATP}^{4,5}. Initial studies using immunoreactivity identified the inward rectifying K⁺-channel subunit Kir6.1 as localizing to mitochondria^{6,7}, and thus Kir6.1 became an attractive candidate as a possible subunit of the mitoK_{ATP}. However, further research by mass spectrometry into the specificity of the Kir6.1 antibody revealed that the antibody does not recognize Kir6.1, and that Kir6.1 is not isolated from more thorough proteomic screens of mitochondria⁸. Another investigation led to purification of an inward-rectifying K⁺-channel component of the mitochondria, but did not identify any specific protein⁹. In a separate study, sulfonylurea receptor (SUR)2 was presented as a possible candidate for a mitoK_{ATP} channel component¹⁰. The long form of SUR2 is needed for the diazoxide-sensitive K⁺ current. The short form of SUR2 localizes to mitochondria, and is able to form

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Disclosures

None

a glibenclamide- and ATP-sensitive K^+ current on the cell surface with Kir6.1. The protective effects of SUR2 have been shown directly only in *E. coli*, and not in cardiac cells. Whether SUR2 is truly necessary for the ATP-sensitive K^+ current in the mitochondria, and whether it plays a role in IPC, remains to be seen. The mitoK_{ATP} has also been proposed to be a multiprotein complex, and succinate dehydrogenase was found to play a regulatory role in mitoK_{ATP} activity¹¹. However, the identity of the protein that is responsible for the K^+ -channel activity in this complex is not known.

The search for mitoK_{ATP} subunits has been complicated by the lack of specificity of pharmacological agents that target the mitoK_{ATP}. Diazoxide, a commonly used drug for mitoK_{ATP} activation, has been shown to activate the sarcolemmal mitoK_{ATP} as well¹². This drug, along with the mitoK_{ATP} inhibitor 5-HD¹³, also affect metabolism and therefore may have a confounding contribution to the protective effects of IPC that is not directly related to the mitoK_{ATP}. Thus, pharmacological manipulation alone has proven to be insufficient in identifying mitoK_{ATP} components.

In this issue of *Circulation Research*, Foster et al. combine a high-throughput proteomic screen with pharmacological and genetic manipulation to provide evidence that renal outer medullary potassium channel (ROMK) is a component of the K^+ channel of the cardiac mitoK_{ATP}¹⁴. The authors employ mass spectrometry analysis of a scaled-up preparation of bovine heart mitochondria to identify ROMK as an inner membrane component, and verify its localization to the mitochondria. They also determine that the ROMK inhibitor Tertiapin Q decreases mitoK_{ATP} activity, and that ROMK knockdown inhibits ATP-sensitive and diazoxide-activated mitochondrial uptake of the K^+ surrogate thallium. Finally, they find that ROMK overexpression protects against cell death in H9c2 cardiomyoblasts, while ROMK knockdown increases cell death.

The findings by Foster et al. are novel and compelling in identifying ROMK as a subunit of the mitoK_{ATP} channel. This study also brings up new questions about the mitoK_{ATP}. First, while the relatively low abundance of ROMK isoforms in the mitochondria presents particular technical difficulties in studying them, it is now pertinent to investigate what other proteins bind to endogenous levels of ROMK. Specifically, given the previous data on SUR2, it would be of interest to see whether endogenous cardiac SUR2 and ROMK can co-precipitate and form functional K^+ channels in the mitochondria (Figure). Additionally, it is unclear whether ROMK2 is the particular ROMK isoform present in the mitoK_{ATP}. More detailed studies on the function of each isoform are needed to determine if there are isoform-specific differences in ROMK localization and K_{ATP} activity. It is also unknown whether ROMK transports K^+ selectively in the mitochondria, or if it is involved in the transport of other ions as well. Moreover, ROMK was chosen as a candidate for further study from the proteomics screen based on its known K^+ -transporter properties. However, additional targets from the screen may also be part of the mitoK_{ATP} channel, even if they are not known to be K^+ transporters. A thorough second look at the results of this screen may reveal other potential candidates for mitoK_{ATP} components.

As many pharmacological agents have been proven to lack specificity for mitoK_{ATP}, research on the mitoK_{ATP} must take care to utilize genetic manipulation in addition to

pharmacological treatment. Foster et al. combined both methods by separately using ROMK knockdown and Tertiapin Q to show inhibition of mitoK_{ATP}. However, in addition to its known inhibition of ROMK, Tertiapin Q may also have nonspecific ROMK-independent effects that contribute to its reduction of mitoK_{ATP} activity. If there is any ROMK-independent mitoK_{ATP} inhibition by Tertiapin Q, identification of the potential nonspecific effects would be needed for future studies utilizing this toxin.

A logical next step to follow up on the results from Foster et al. is to investigate whether ROMK confers mitoK_{ATP} activity *in vivo* as well. ROMK knockout or transgenic animals should now be studied for mitoK_{ATP} activity as well as IPC protection. These experiments would be important in determining whether ROMK is a physiologically relevant factor of mitoK_{ATP} and IPC *in vivo*.

In summary, the components of the mitoK_{ATP} have remained elusive since its initial discovery, as investigation into its composition has been hindered by the low abundance of K⁺ transporters in the mitochondria as well as a lack of pharmacological specificity for the mitoK_{ATP}. Foster et al. now demonstrate that ROMK is a novel component of the mitoK_{ATP} through the combination of a relatively large and specific proteomic screen with pharmacological and genetic techniques. Future studies will reveal what other proteins bind to ROMK, whether there are ROMK isoform-specific differences in mitoK_{ATP} function, and whether this protein is a part of the *in vivo* mitoK_{ATP}.

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Non-standard abbreviations

IPC	Ischemic preconditioning
SUR	sulfonylurea receptor
ROMK	renal outer medullary potassium channel

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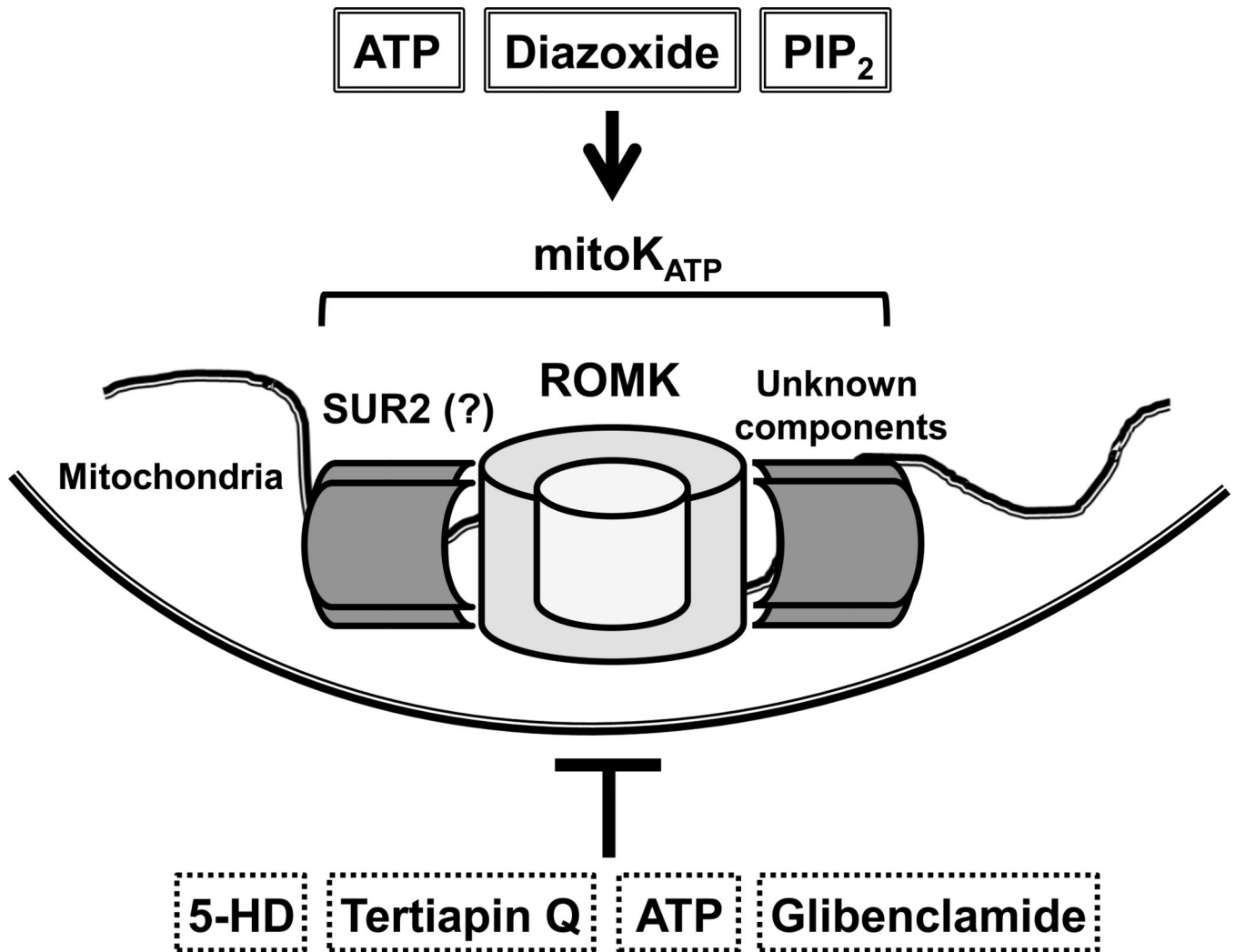


Figure. Proposed MitoK_{ATP} structure

A proteomic screen of inner mitochondrial membrane components identified ROMK as a protein possessing mitoK_{ATP} channel activity which responds to known regulators of the mitoK_{ATP} channel, such as the pharmacologic activator diazoxide and the inhibitor 5-hydroxydecanoate (5-HD). While not established, ROMK may interact with another proposed component of the mitoK_{ATP} channel, the short splice variant of sulfonylurea receptor 2 (SUR2).