Published in final edited form as:
Pflugers Arch. 2014 May ; 466(5): 875–886. doi:10.1007/s00424-013-1359-0.

MaxiK channel and cell signalling

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Abstract

The large-conductance Ca^{2+}- and voltage-activated K^+ (MaxiK, BK, BK_{Ca}, Slo1, $K_{Ca}1.1$) channel role in cell signalling is becoming apparent as we learn how the channel interacts with a multiplicity of proteins not only at the plasma membrane but in intracellular organelles including the endoplasmic reticulum, nucleus and mitochondria. In this review, we focus on the interactions of MaxiK channels with seven transmembrane G-protein coupled receptors, and discuss information suggesting that the channel big C-terminus may act as nucleus of signalling molecules including kinases relevant for cell death and survival. Increasing evidence indicates that the channel is able to associate with a variety of receptors including β-adrenergic receptors, G-protein coupled estrogen receptors, acetylcholine receptors, thromboxane A2 receptors and angiotensin II receptors, which highlights the varied functions that the channel has (or may have) not only in regulating contraction/relaxation of muscle cells or neurotransmission in the brain but also in cell metabolism, proliferation, migration and gene expression. In line with this view, MaxiK channels have been implicated in obesity and in brain, prostate, and mammary cancers. A better understanding of the molecular mechanisms underlying or triggered by MaxiK channel abnormalities like overexpression in certain cancers may lead to new therapeutics to prevent devastating diseases.

Keywords

BK channel; BK_{Ca} channel; Slo1; $K_{Ca}1.1$; macromolecular complexes; protein-protein interactions; human pathology

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MaxiK channel distinctive characteristics

MaxiK channels, also known as BK/BK_{Ca}/Slo1/K_{Ca}1.1 channels, are encoded by the KCNMA1 gene and characterized by a large conductance to potassium, their sensitivity to voltage and Ca^{2+} and ubiquitous expression. In addition to responding to changes in Ca^{2+} and voltage, MaxiK channels also sense gases, lipids, and associate with a multiplicity of plasma membrane and intracellular proteins being linkers of membrane potential, cell metabolism, and cell signalling [21, 41, 70].

MaxiK channel essential structure consists of four α-subunits, each formed by 7 transmembrane segments and a large C-terminus that constitutes about two thirds of the protein [46] (Fig. 1). This tetrameric structure can be complemented with regulatory subunits. In mammals, the auxiliary subunits, β1-β4 and the recently discovered, γ1-γ4, can greatly modify channel performance including its response to pharmacological agents, kinetics, and Ca^{2+}/voltage sensitivities. For example, β4 makes channels resistant to iberiotoxin blockade [47], γ1 (also named LRRC26) makes channels resistant to mallotoxin activation [3], β2 produces MaxiK channels that inactivate with time [84], β3b produces channels with very fast inactivation producing currents that appear to activate fast and rectify [81, 89], β1 increases Ca^{2+} /voltage sensitivity when free Ca^{2+} facing the inside of the channel is beyond 1 μM [45], while γ1-γ4 produce channels with increased voltage sensitivity even without Ca^{2+} [91, 92]. Functional diversity of MaxiK channels is also conferred by alternative splicing of both α- and β-subunit mRNAs and by posttranslational modifications like phosphorylation and lipidation [1, 76, 99].

In spite of its ubiquitous expression, the genetic ablation of MaxiK α-subunit in mice was not lethal indicating that animals developed compensatory mechanisms to substitute for MaxiK potential vital function in organs where it is normally expressed and/or that MaxiK is not essential to sustain life but rather it may serve to fine tune numerous body functions. In line with this view, more and more reports show MaxiK multiple physiological roles and involvement in a wide variety of diseases. In this respect, the lack of the α-subunit produces body deficiencies that, although not fatal, on the long run, decrease the quality of life. For example, mice lacking the α-subunit suffer from incontinence, erectile dysfunction, hypertension, altered circadian rhythm, and age-dependent hearing deficiencies [48, 49, 62, 63, 86].

MaxiK cellular compartmentalization

Immuno-mapping or electrophysiological methods indicate that in most cells MaxiK channels are expressed at the plasma membrane with adult cardiomyocytes being an interesting exception. In addition to its plasma membrane targeting, MaxiK channels can also localize to intracellular organelles like the endoplasmic reticulum, nucleus and mitochondria (Fig. 2) [67, 68, 70, 90]. The latter opens the tantalizing possibility that MaxiK channels might play a role in regulating mitochondria function and consequently cell death.

What are the intrinsic MaxiK signals targeting the channel to different cellular compartments or organelles? This is an active line of research. Currently it is known that there are several signals within the channel backbone that serve as different checkpoints to

deliver the protein to the plasma membrane; three of them are located after the Ca^{2+}-bowl in the regulator of conductance for K^+ (RCK) 2 domain and another is located in the linker of RCK1 and RCK2 domains (Fig. 1, double arrows). The former contains dihydrophobic motifs (YGDLFCKALK; YNMLCFGI, which is also a caveolin-1 interaction site; and DLIFCL) known to act as endoplasmic reticulum export signals [2, 5, 31, 88]; while the latter contains an acidic cluster DDITDPKRI important for trafficking of other potassium channels [11, 44]

In addition to intrinsic signals in the constitutive MaxiK protein, splice variation of the α-subunit can add or delete signal sequences modifying the channel localization by facilitating its retention/targeting to intracellular organelles like the endoplasmic reticulum [11, 26, 43, 97, 98] and mitochondria [68]. A current challenge is to correlate the expression of specific splice variants to functional effects in native systems. In this respect, a 33 aa insert in transmembrane domain 1 containing CVLF trafficking signal that retains MaxiK in the endoplasmic reticulum of HEK293T cells [97, 98] is increased in rat aging corpora decreasing the channel surface expression [14]; this property may contribute to aging-related changes in male sexual activity. Another example is a 27 aa encoding exon, named ALCOREX (Fig. 1), which produces channels in HEK293 cells that develop a transient sensitivity to alcohol that is higher and more persistent than the one observed for the insertless channel. Importantly, MaxiK channel activation by alcohol decays in ~10 min which correlates with decreased ALCOREX expression in hypothalamo-neurohypophysial system explants exposed to alcohol [57]. This 27 aa insert also confers MaxiK channels its ability to be activated by arachidonic acid in growth hormone secreting neurons, GH3 cells [34].

Interestingly, intron containing cytoplasmic mRNAs can also contribute to differences in channel expression levels and electrical activity in hippocampal neurons. This regulatory mechanism seems to provide a way for specific and local expression of MaxiK variants containing the 59 aa splice insert, STREX (Fig. 1) [7, 8]. Another regulatory mechanism for splice variant expression is via microRNA-9 (miR9), which can be upregulated by exposure to alcohol, immunodeficiency virus and methamphetamines in the brain [57, 73].

Membrane receptors and MaxiK channels

Biologically active substances like hormones, peptides, and lipids bind to membrane receptors triggering the activation of signalling molecules, many of which are kinases and phosphatases, to regulate multiple cellular events like contraction/relaxation, cell proliferation, migration and gene expression.

MaxiK channels are known to be functionally coupled to a variety of plasma membrane receptors like β-adrenergic receptors, ACh receptors, thromboxane A2 receptors, angiotensin II receptors, and to the G-protein coupled estrogen receptor 1. The functional coupling between MaxiK channels and membrane receptors usually uses intermediary proteins as G-proteins and protein kinases that serve to transduce the signals received by the receptor to induce changes in channel activity which can be inhibitory or excitatory depending on the receptor being stimulated or the hormonal status of the tissue. Recently, we uncovered a new

transduction mechanism that utilizes direct protein-protein interactions between the Thromboxane A2 receptor and the MaxiK channel that is independent of G-protein activation and results in channel inhibition ("trans-inhibition") [35].

The coupling between membrane receptors and MaxiK channels has been mostly studied in smooth muscle where muscle relaxants like β-adrenergic agents and estrogen induce MaxiK channel activation and constrictors like Acetylcholine, Thromboxane A2, and Angiotensin II result in channel inhibition.

β2-adrenergic receptor

Early studies using bilayers and smooth muscle membranes showed that, after biochemical reconstitution, MaxiK channels can remain associated with β-adrenergic receptors in complex with G-proteins pointing to stable and multi-protein interactions [64, 80]. Indeed, association of β2-adrenergic receptors and MaxiK channels has been demonstrated in brain where they form a large macromolecular complex that includes protein kinase A (PKA), cytosolic A-kinase-anchoring protein (AKAP79/150), and the L-type Ca^{2+} channel [38].

Signalling mechanisms triggered by β2-adrenergic stimulation coupled to MaxiK activation have been studied in smooth muscle reconstituted in lipid bilayers and native cells, and involve a membrane-delimited action of the α-subunit of Gs ($G_s\alpha$) on MaxiK channels as well as protein kinase A mediated phosphorylation of the channel protein [27, 28, 51, 64].

At the molecular level, the target site for PKA-mediated phosphorylation and activation of MaxiK channels is located in the RCK2 domain (^{866}RQPS*^{869}, numbers are as in GenBank U11058) [51]. All four MaxiK α-subunits need to be phosphorylated at this site for the channel to be activated [76]. Evidence for a direct action of PKA on the channel protein, is its ability to associate with MaxiK channels in the brain and the finding of the corresponding MaxiK phospho-peptide by proteomic analysis of immunopurified MaxiK also from the brain [38, 93]. Whether $G_s\alpha$ interacts directly or forms a complex with MaxiK channels during channel activation is yet to be determined.

Interestingly in non-pregnant myometrium, PKA can also cause inhibition of the MaxiK channel [56] but the triggering membrane receptor/mechanism of this response is unknown. The molecular correlate of the PKA-inhibited MaxiK channel is a channel isoform that contains the STREX insert, which introduces an additional site for PKA dependent phosphorylation [77]. Only a single STREX containing MaxiK α-subunit is required for PKA-dependent inhibition of channel activity but the constitutive S^{869} site must be dephosphorylated. Importantly, STREX is more abundant in non-pregnant myometrium and decreases with estrogen levels or pregnancy [102] explaining why, in non-pregnant myometrium, the majority of MaxiK channels are inhibited by PKA whereas during mid-pregnancy they are activated [56] probably contributing to the maintenance of uterine quiescence for a successful pregnancy.

G-protein coupled estrogen receptor 1

The G-protein coupled estrogen receptor 1 (GPER1) is a recently discovered seven transmembrane receptor that is activated by estrogen. GPER1 plays a role in protecting the

myocardium from ischemic insult by the activation of salvage kinase pathways and prevention of mitochondrial dysfunction [10, 15, 52]. Using its agonist G1, it has been shown that in coronary smooth muscle, GPER1 stimulation results in an increased MaxiK channel activity. This increased channel activity correlates with relaxation of precontracted vessels by G1, which can be prevented by iberiotoxin, a MaxiK channel blocker [95]. Whether GPER1 and MaxiK channels interact closely with each other and/or the identification of signalling pathways used by GPER1 to modulate MaxiK channel activity are still open questions.

Muscarinic acetylcholine receptor M2

Initial evidence for acetylcholine receptor Gi-mediated inhibitory coupling to MaxiK channels was given in ileum and tracheal myocytes [13, 29]. However, a positive regulation has also been observed in canine tracheal myocytes [82] perhaps due to experimental conditions lacking GTP for proper coupling with G proteins. Although PKC dependent inhibition of MaxiK channels is well known, its role as an inhibitory pathway induced by muscarinic M2 receptors was only addressed recently. In HEK cells as well as in tracheal myocytes, M2-mediated inhibition of MaxiK channels involves two mechanisms; i) a membrane delimited Gβγ mediated inhibition, and ii) phospholipase C/PKC activation. Purified transducin Gβγ-mediated inhibition of MaxiK channel activity occurs independently of activation of PLCβ isozymes, intracellular Ca^{2+} concentration, or expression of MaxiK β1-subunit. Further, Gβγ can associate with MaxiK channels as it is possible to coimmunoprecipitate all proteins in HEK cells expressing the recombinant proteins [100]. PKC phosphorylation of MaxiK channel α-subunit occurs in the C-terminus at consensus PKC sites ^{642}S*PKKK^{646} and ^{1097}KS*R^{1099} with only one channel subunit requiring phosphorylation for channel inhibition [101]. Phosphorylation at ^{642}*S but not ^{1098}*S was detected by proteomic analysis due to missing sequence information in this region [93].

Thromboxane A2 and Angiotensin II receptors

Thromboxane A2 prostanoid receptor (TPR) and Angiotensin II type 1 receptor (AT1R) are G-protein coupled receptors (GPCR) that play important roles in the development of vascular diseases such as heart angina, hypertension and stroke due to the potent vasoconstrictor effects of their agonists, Thromboxane A2 (TXA2) and Angiotensin II (Ang II). In models of chronic vascular disease (e.g. hypertension, aortic regurgitation, atherosclerosis), TPR and AT1R gene ablation ameliorate disease symptoms (i.e. reduces blood pressure, cardiac hypertrophy, age-related progression of atherosclerosis) underscoring TPR and AT1R role in the pathogenesis of vascular disease and end-organ injury [17–19]. The functional interaction between these receptors and MaxiK channels was first made evident in experiments performed in lipid bilayers using coronary smooth muscle membrane vesicles, where thromboxane A2 as well as angiotensin II produced the inhibition of channel activity [65, 79]. The inhibition of MaxiK channel activity by angiotensin II presumably via AT1R was later observed in coronary myocytes [42]. Interestingly, in lipid bilayers the inhibition of channel activity occurred without the addition of GTP suggesting the involvement of a G-protein independent mechanism(s) [65, 79]. In line with this view and as mentioned earlier, we recently demonstrated that in fact TPR trans-inhibits MaxiK

channels independent of G-protein activation and via a mechanism that likely involves direct protein-protein interaction(s) between the receptor and the α-subunit of the channel. The MaxiK-TPR association utilizes the voltage-sensing-conduction cassette of the channel α-subunit and the first intracellular loop and C-terminus of the receptor [35]. Figure 2A–C shows the colocalization of TPR and MaxiK channel α-subunit at the cell surface of human coronary myocytes.

The MaxiK channel-TPR association also includes the channel β1 regulatory subunit. Interestingly, the β1-subunit can independently associate with the receptor and the channel α-subunit leading to the interesting hypothesis that β1-subunit could alter TPR function. Supporting this view, we found that β1 gene ablation produces blood vessels with twice the sensitivity to thromboxane A2, i.e. aortic strips showed an EC_{50} to thromboxane A2 agonist, U46619, of 18 nM in the wild type animals and an EC_{50} of 9 nM in the β1 null mice [36]. Whether the β1-subunit also associates with other G-protein coupled receptors, modifying their vasoconstricting/vasorelaxing potencies remains open to research. Also, a detailed study of how angiotensin II modifies MaxiK channel activity and whether AT1R is in close contact with the channel are topics that need scrutiny.

MaxiK and cell signalling

The coupling of MaxiK channels with 7 transmembrane receptors described above necessarily link MaxiK channels with cell signalling events, like activation of PKA and PKC. Moreover, direct protein-protein interactions have been found between the channel and focal adhesion kinase (FAK), and cytosolic phospholipase A2 (cPLA2), as well as regions in MaxiK channel necessary for association with PKA complexes and spleen tyrosine kinase (SyK) (Fig. 1 and Table 1). This information in combination with recent proteomic data indicates that aside from its role in K^+ conduction, MaxiK channel may provide surface contact for a variety of proteins. In this view, MaxiK channel could act as "coordinator" or "linker" of signalling events.

Back in 2006, our review listed near 20 proteins that had been recognized as MaxiK α-subunit partners including plasma membrane and cytosolic proteins [41]. Today this list has grown to the hundreds thanks to the establishment of the proteomics technology which has revealed that MaxiK channel is in complex with proteins not only at the plasma membrane or cytosol but also with proteins in organelles like the endoplasmic reticulum, nucleus and mitochondria [24]. Interestingly, this approach has revealed the association of MaxiK channels with kinases relevant for cell death and survival, like Akt, glycogen synthase kinase-3β (GSK-3β) and phosphoinositide-dependent kinase-1 (PDK1) opening the intriguing possibility that MaxiK channels are also operators of cell life and dead. In addition, proteins that associate with MaxiK channels also include proteins linked to other cellular processes like metabolism, development, traffic, transport and apoptosis (Figure 3) [71].

Many protein partners of MaxiK α-subunit have been detected/confirmed by coimmunoprecipitation, including Akt, GSK-3β, PDK1, Src, β2-adrenergic receptors, transient receptor potential canonical 1 (TRPC1), and its modulatory β1- and γ1-subunits

among others [30, 41, 71, 91]. In these cases, a direct protein-protein interaction has not been established and, thus indirect interactions cannot be strictly ruled out. Table 1 summarizes those proteins where direct protein-protein interactions have been reported or where MaxiK regions critical for protein associations have been identified. Although many of these interactions may be conserved in different cell types, a big challenge is to determine where, how and when MaxiK channels interact with specific proteins for cell-specific functions and how failures in these interactions may lead to disease.

New views in MaxiK channel physiology and human disease

The majority of studies have been directed to elucidate the physiological role of MaxiK channels expressed at the plasma membrane. However, MaxiK channels are also expressed in intracellular organelles like the mitochondria (Fig. 2D–G), where their physiological role is beginning to be understood [24, 70]. These advances together with the linkage of MaxiK channel defects to human disease are highlighted below.

Role in mitochondria—In cardiac mitochondria, pharmacological evidence indicates that MaxiK channel opening improves mitochondrial respiratory function and protects the heart from ischemic insult [4, 90]; in mitochondria from an astrocytoma cell line, their electrical activity is coupled to the respiratory chain [6], and in pulmonary artery smooth muscle, 11,12-epoxyecidosatrienoic acid induced vasoconstriction and mitochondrial depolarization has been linked to mitochondrial MaxiK (mitoBK$_{Ca}$) and its association with its β1-subunit [40].

The molecular correlate of mitoBK$_{Ca}$ was unknown until recently. As expected from its electrophysiological properties, we found that mitoBK$_{Ca}$ is encoded by the same gene (*Kcnma1*) encoding its plasma membrane counterpart and is formed by α-subunits of about 140 kDa. A C-terminal spliced exon (named after the 3 last aa DEC) (Fig. 1) is required for the channel mitochondrial targeting. Using the MaxiK α-subunit knockout animal, we also confirmed that opening of these channels with NS1619 protects the heart from ischemic insult. Mechanisms underlying this protection are enhanced performance of mitochondria and cardiac neurons [68, 87]. These findings together with the fact that MaxiK channels associate with the salvage kinase Akt and with GSK3β [71] an integrator of signals whose phosphorylation prevents mitochondrial permeability transition are strong arguments in favor of a role of MaxiK channels in cardioprotection and support a possible role in cell survival.

Role in human disease—During the last decade, genetic studies in humans have revealed mutations and genomic amplification of MaxiK channel gene leading to a variety of diseases involving the brain, metabolism, and cell proliferation/migration.

i. Brain disease. Paroxysmal movement disorder and generalized epilepsy was the first disorder correlated to a mutation in MaxiK channels. The mutation within the RCK1 domain neutralizes a negatively charged residue (D369-G). Out of 13 individuals affected with the disease, all carried the neutralizing mutation, which produces channels with higher sensitivity to Ca^{2+} in heterologous expression. This feature would accelerate the repolarization of action potentials and explain an

increase in neuronal firing rate in the disease [16]. MaxiK channel dysfunction has also been associated with autism and mental retardation. These maladies have been related, in one case, to a chromosomal translocation event that resulted in silencing of one copy of the gene and thus, reduced MaxiK expression; while in another case a conserved mutation (A73-V) was found in the first intracellular loop [32].

ii. Metabolic disease. Genome-wide association analyses identified the MaxiK channel gene as linked to human obesity in 5 from 6 case-control cohorts (total of 4214 obese vs. 5417 lean individuals). Moreover, MaxiK transcript expression was increased in adipose tissue and isolated fat cells from obese individuals [22]. These findings open a new line of research to understand the metabolic pathways linked to MaxiK expression and function.

iii. Cardiovascular disease. Severe hypertension has been linked to a polymorphism in an intronic sequence of MaxiK channel gene, and a haplotype with an additional polymorphism (in the fourth exon, C864T) has been linked to increased risk of myocardial infarction in addition to systolic and general hypertension. However, the C864T haplotype corresponds to a synonymous single nucleotide polymorphism Phe229Phe, and the polymorphism in the intronic sequence did not generate a MaxiK channel isoform. Although there seems to be no functional effects, these mutations may serve as genetic markers of increased risk for cardiovascular disease [78]. In line with the view that the MaxiK channel itself is not modified in human hypertension, a recent report from Chinese patients with hypertension show that this was the case; instead in this population, MaxiK channel regulatory β1-subunit was the one that was reduced causing the expected decrease in the channel Ca^{2+} sensitivity and voltage-dependence of activation [94].

iv. Cancer. MaxiK channel overexpression has been correlated with the malignancy of human gliomas; accordingly, its inhibition reduced glioma cell growth. A key factor in the development of glioma seems to be a distinctive 34 aa splice variant insert (Fig. 1, arrow, sequence in red), which to our knowledge has not been detected in healthy cells. This 34 aa insert is in tandem with a previously reported 29 aa spliced exon (Fig. 2, arrow, sequence in gray) producing channels with higher Ca^{2+} sensitivity [39, 85]. The glioma MaxiK isoform (gBK) is expressed at the cell membrane, mitochondria, Golgi, and endoplasmic reticulum and is also found in other types of tumor cell lines derived from duodenal, colon, hepatocellular and pancreatic cancers. Importantly, this molecular information has provided the tools to generate gBK peptide-specific cytotoxic T lymphocytes and kill cells expressing gBK [20].

Amplification of MaxiK channel gene is another mechanism that has been correlated with cancers; specifically with prostate and breast cancers. Obviously, it is important to elucidate the mechanisms triggered by this genomic amplification as breast cancers associated with MaxiK gene amplification are those of high tumor grade, high cell proliferation and poor prognosis [9, 53]. Moreover, it would be relevant to determine the molecular composition of MaxiK in breast cancer cells and to determine whether it contains the gBK isoform.

Concluding Remarks

From the stand point of cell signalling, it is intriguing the multitude and variety of molecules that can form complexes with MaxiK channels underscoring the necessity to understand the dynamics of these connections and their physiological consequences. One highlight in this regard, is the interaction of MaxiK channels and G-protein coupled receptors with opposite functions in smooth muscle, relaxation and constriction. When do these interactions take place and are they affected in disease or during aging are few of the questions that would be important to address in the future. At the molecular level, the soluble C-terminus of the channel α-subunit represents two thirds of the protein, and thus, may provide a significant surface contact for direct protein-protein interactions. Future studies need to identify which of the hundreds of proteins found by proteomics to associate with MaxiK channel α-subunit, may serve as scaffolds directly binding to the channel and thus, facilitating the association with other proteins and/or relay of signals to other signalling clusters.

Finally, the increasing evidence about the role of MaxiK channels in human disease including obesity and cancer offer opportunities to understand the mechanisms of human disease and design new strategies for their potential cure.

Acknowledgments

This work was supported by NIH grants, HL107418 and HL096740 (LT, ES), and HL088640 (ES), and by the American Heart Association National Scientist Development Award 11SDG7230059 (HS). Kcnma1−/− was kindly provided by Dr. Andrea Meredith (University of Maryland School of Medicine).

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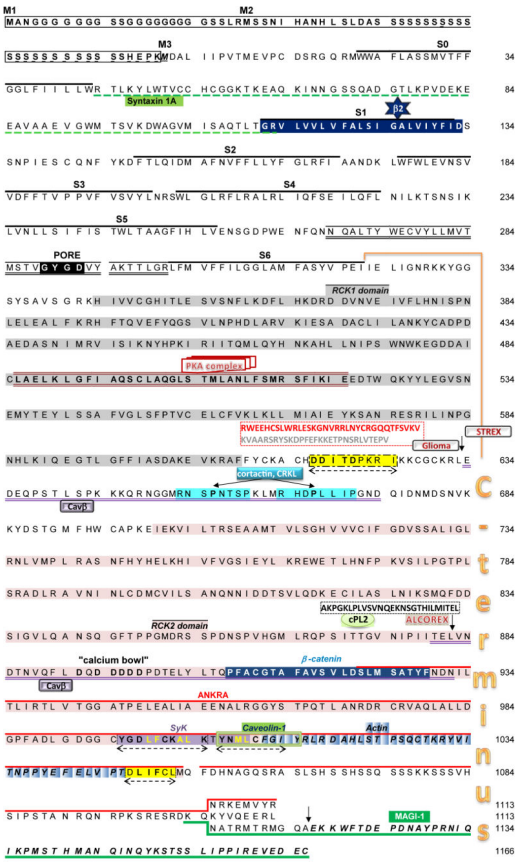

Figure 1. MaxiK α-subunit sequence: interacting regions and protein partners

Amino acid sequence ending with QEERL corresponds to “insertless” hSlo; numbering starts at the third Methionine (M3) (NCBI # U11058). Highlighted are 3 possible start sites M1–M3 [83], the 7 transmembrane domains S0–S6, the pore region, the whole C-terminus, the regulator of K^+ conductance (RCK) domains 1 (RCK1; gray) and 2 (RCK2; pink), the calcium bowl, export signals (dashed double arrows, yellow), and examples of regions reported to date to be involved in direct or indirect protein interactions. For a complete list of interacting proteins and references see Table 1. For clarity, interacting sequences in Table 2 that include large portions of the protein were omitted in this figure. Arrows mark the position of the glioma-specific 33 aa splice insert (red) upstream a 29 aa splice insert (gray), of the 59 aa splice insert encoded by the STREX exon, of the 27 aa splice insert known as ALCOREX [57], and of the 50 aa C-terminal exon named after the last three amino acids, DEC. Three C-terminal isoforms are shown; the sequences of the DEC containing isoform correspond to the mouse brain isoform, mbr5 [66]. For the crystal structure of the C-terminus, see [96].

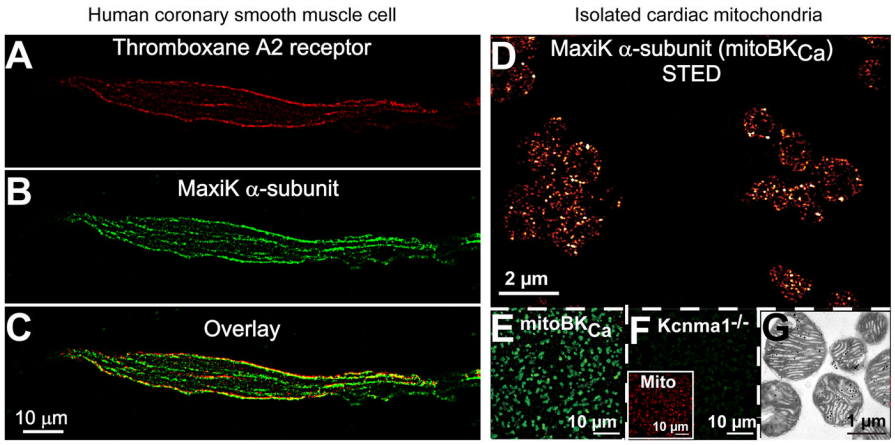

Figure 2. MaxiK α-subunit expression at the plasma membrane in smooth muscle cells and in cardiac mitochondria

A–C. Freshly dissociated human coronary arterial myocyte double-labeled with anti-MaxiK monoclonal **(A)** and anti-thromboxane A2 receptor polyclonal (**B**) antibodies. The overlay **(C)** shows co-localization of both proteins at the plasma membrane. **D.** Super-resolution fluorescence images of Percoll-purified cardiac mitochondria [69] labeled for endogenous MaxiK α-subunit (mitoBK$_{Ca}$) with anti-MaxiK polyclonal antibody as described in [68]. Note distinct punctae of mitoBK$_{Ca}$ clusters. Images were acquired with a custom-made stimulation emission depletion (STED) microscope and pseudocolored for presentation. **E–F.** Low resolution confocal fluorescence images of purified mitochondria showing the specificity of the anti-MaxiK antibody (green) with only background signals in mitochondria from the knockout animal (Kcnma1$^{-/-}$). The inset in F shows the same field double labeled with mitotracker (red). **G.** Electron micrograph of the purified mitochondria preparation. Images were acquired at 0.0575 μm/pixel in **(A–B, E–F)** and at 0.0035 μm/pixel in **(D)**, and were median filtered to reduce non-specific background as described earlier [36].

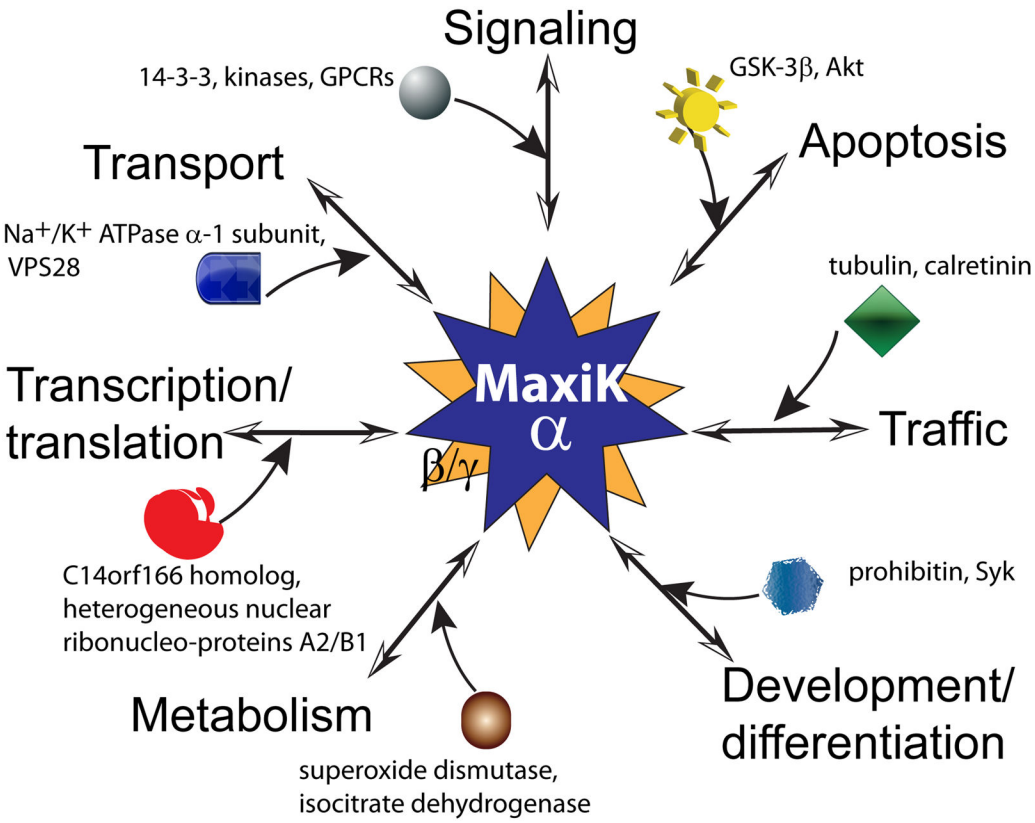

Figure 3. MaxiK channel potential functions uncovered by associated protein partners

Proteomic analyses have revealed that MaxiK channels not only associate with β and γ regulatory subunits but also with a large variety of proteins involved in diverse cellular functions [24, 71]. Note that proteomics cannot differentiate between direct (binding to the channel) or indirect interactors. We hypothesize that MaxiK α subunit association with some proteins may be relatively steady like with regulatory α and γ subunits, while association with other partners (symbols) could be dynamic (incoming arrows); in both cases, interactions may be tissue-specific and distinct depending on gender, age and health-status among others. Examples of MaxiK associated proteins known to be involved in: 1) signaling are 14-3-3 proteins, visinin-like protein 1 [71], G-protein coupled receptors [35]and kinases like PKA complex [75], and Akt (protein kinase B); 2) traffic are tubulin [54], calretinin [71], the small GTPase Rab11b [72], actin [103]; 3) development/differentiation are prohibitin, Thy-1 membrane glycoprotein precursor [71], Syk [59]; in transcription/ translation are protein C14orf166 homolog and heterogeneous nuclear ribonucleo-proteins A2/B1 [72]; 4) metabolism are mitochondrial precursors of fumarate hydratase and superoxide dismutase [Mn], isocitrate dehydrogenase [71]; 5) apoptosis are glycogen synthase kinase-3β (GSK-3β) and Akt [71]; and 6) transport are Na^+/K^+ ATPase alpha-1 subunit, vacuolar protein sorting associated protein 28 homolog (VPS28) [71].

Table 1

MaxiK α subunit domains and protein complexes

Interacting proteins	MaxiKα interaction/association motif	Method	Co-IP or co-labeling	Ref
	Transmembrane proteins			
Direct Interactions				
β2-subunit	S1 transmembrane domain (aa ^{112}GRVL--YFID^{133}, #U11058)	prokaryote two-hybrid system	in vitro	[50]
Thromboxane A2 receptor	voltage-sensing-conduction cassette including N-terminus (aa ^{1}MDAL---YVPE^{321}, #U11058)	FRET	coronary smooth muscle	[35]
	Intracellular proteins			
Direct interactions				
FAK	Whole C-terminus (^{388}IELI---EERL^{1178}, #U13913)	yeast two-hybrid system	osteosarcoma cells (MG63)	[58]
Microtubule-associated protein1A	partial C-terminus (aa 746–1144*)	yeast two-hybrid system	brain, Purkinje cells	[55]
β-catenin	"S10" hydrophobic segment (^{941}PFAC---TYF^{962}, numbers as in [61])	yeast two-hybrid system	chicken hair cells	[33]
Cereblon	Partial C-terminus (encompassing RCK1 and part of RCK2 domains upstream the Ca^{2+}-bowl; aa 394–955 of rSlo*)	yeast two-hybrid system	brain, hippocampal neurons	[23]
ANKRA	C-terminal end (downstream the Ca^{2+} bowl, aa ^{1019}SLM--MVYR^{1210}, #AF135265.1)	yeast two-hybrid system	brain	[37]
Actin	FGIYRLRDAHLSTPSQCTKRYVITNPPYEFELVPT	purified proteins	chick ciliary ganglion	[103]
cPLA2	AKPGKLPLVSVNQEKNSGTHILMITEL (in 27 aa splice insert or ALCOREX)	mammalian two-hybrid system	GH3 cells	[34]
Rab11b	region excluding N- and C-terminus	yeast two-hybrid system	chick cochlea	[72]
Cortactin, CRKL	^{656}P and ^{667}P in RxxPxxxP proline rich motifs	overlay assay	brain	[74]
MAGI-1	C-terminus DEC variant (1111–1171*); sequence in Fig. 2 is as in [43]	yeast two-hybrid system	podocyte cell line	[60]
Ca_vβ1	C-terminus fragments including Ca^{2+} bowl (884T–N936) or non-canonical SH3 binding domain (E637–D677)*	yeast two-hybrid system and purified proteins	chick ciliary ganglion neurons	[104]
Direct/Indirect?				
Nephrin	VEDEC variant*	GST pull-down assays	podocyte cell line	[25]
Syntaxin 1A	S0–S1 loop/C-terminus (^{336}YSAVSG----VEDEC^{1166}; numbers as in Fig. 2)	co-IP	brain	[12]
Caveolin-1	Caveolin binding motif, YNMLCFGIY	co-IP	aorta	[2]
Tubulin	RCK2 to C-terminal end (^{679}MDS---QEERL^{1113}, #U11058)	pull-down with purified protein	astrocytes	[54]
PKA complex (PKA indirect)	leucine zipper 1, LAELKLGFIAQSCLAQGLSTMLANLFSMRSFIKIE	co-IP	brain	[75]
SyK	ITAM, YGDLFCKALKTYNML	co-IP	osteosarcoma cells(MG63)	[59]

aa, amino acid; ANKRA, Ankyrin-repeat family A protein; co-IP, co-immunoprecipitation; cPLA2, cytosolic phospholipase A2; CRKL, Crk-like protein; FAK, focal adhesion kinase; ITAM, immunoreceptor tyrosine-based activation motif; MAGI-1, membrane-associated guanylate kinase with inverted orientation protein-1; PKA, protein kinase A; SH3, Src homology 3; ?, in these cases a direct or indirect interaction has not been demonstrated; #, NCBI accession number; *, NCBI accession number not given (amino acid numbers are as given in publications and may not coincide with template used in Fig. 2)