Physiology of intracellular potassium channels: A unifying role as mediators of counterion fluxes?☆

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ABSTRACT

Plasma membrane potassium channels importantly contribute to maintain ion homeostasis across the cell membrane. The view is emerging that also those residing in intracellular membranes play pivotal roles for the coordination of correct cell function. In this review we critically discuss our current understanding of the nature and physiological tasks of potassium channels in organelle membranes in both animal and plant cells, with a special emphasis on their function in the regulation of photosynthesis and mitochondrial respiration. In addition, the emerging role of potassium channels in the nuclear membranes in regulating transcription will be discussed. The possible functions of endoplasmic reticulum-, lysosome- and plant vacuolar membrane-located channels are also referred to. Altogether, experimental evidence obtained with distinct channels in different membrane systems points to a possible unifying function of most intracellular potassium channels in counterbalancing the movement of other ions including protons and calcium and modulating membrane potential, thereby fine-tuning crucial cellular processes. This article is part of a Special Issue entitled ‘EBEC 2016: 19th European Bioenergetics Conference, Riva del Garda, Italy, July 2–7, 2016’, edited by Prof. Paolo Bernardi.

1. Introduction

Potassium channels are widely spread over all kingdoms, ranging from viruses to humans, but the physiological roles they play in the different organisms are not well defined for all of them. A characteristic feature of highly selective potassium channels is that their preference for potassium is defined by a conserved amino acid motif, TVGYGD, in the narrowest stretch of the pore known as the selectivity filter [1,2]. Thus, in principle, a simple bioinformatic analysis can be exploited to identify putative potassium channels in different organisms with completely sequenced genomes (see e.g. [3] for prokaryotes). However, one has to keep in mind that several other channels, which allow the flux not only of potassium but of other cations as well, do not display this signature sequence. Yet, under physiological conditions potassium may be the main cation flowing through them. Notable technical advancement in biophysics and cell biology during the last decades has led to the discovery of intracellular potassium channels operating in organelles or in endomembrane systems, even though technical challenges in studying these channels still make their complete characterization difficult: for example ion composition (nature and activity of the ions that are present) on the “extra-cytoplasmic” side of the organelles or the actual membrane potentials are not always known [4]. Surprisingly, the presence and function of several K+ channels known to be located in the plasma membrane (PM) has been demonstrated in various, in some cases even in multiple intracellular membranes as well. Identification of the genes encoding some of these K+ channel activities has allowed researchers to obtain genetic evidence regarding both the localization and the patho(physiological) importance of these non-PM K+ channels. In other cases, determination of their function relies on (often less clear-cut) pharmacology. In the present review we give and updated overview of the growing number of various potassium channels present in intracellular membranes both in animal and plant cells and discuss their possible functions and the open questions. Fig. 1 summarizes the currently known localization of these K+ channels both in plant and animal cells.

2. Mitochondrial potassium channels in animals

Bioenergetics has become central to our understanding of pathological mechanisms as well as the development of new therapeutic strategies and as a tool for gauging disease progression in neurodegeneration, diabetes, cancer and cardiovascular disease. The view is emerging that inner mitochondrial membrane (IMM) K+ channels have a profound effect on mitochondrial function and, consequently, on the metabolic
state and survival of the whole cell. IMM channels in general are beginning to emerge as promising oncological targets as well[5]. Disruption of the sustained integrity of mitochondria is strongly linked to human disease, and the observed cytoprotective effect of certain IMM K+ channels operating in the IMM. Given that IMM K+ channels are encoded by the nuclear genome, the molecular identities of the observed channel activities are still under debate in many (but not all) cases, as are the mechanisms targeting these proteins to multiple sites within the cell. IMM K+ channels recorded by patch clamp include calcium-dependent channels. Big conductance calcium-dependent potassium channel (BKCa)[9], Intermediate-conductance calcium-dependent K+ channel (IKCa) [10] and Small conductance calcium-dependent K+ channel (SKCa)[11], voltage-gated shaker type K+ channel Kv1.3[12] and the ATP-dependent potassium channel (mitoKATP)[13]. For this latter channel, the renal outer medullary K+ channel ROMK2 (Kv1.5) has been proposed to be the channel-forming subunit[14], however the molecular components of mitoKATP are still debated (for recent reviews see[15,16]). Biochemical evidence indicates the presence of the two-pore potassium channel TASK-3[17], of Kv1.5[18], of ROMK2 and of Kv7.4[19] as well. Basically all above-mentioned IMM K+ channels identified thus far are considered to be the mitochondrial counterparts of well-known plasma membrane (PM) channels and many of them display even multiple subcellular localizations. For example, SKCa has been found to be located in PM, mitochondria and ER as well, where it is essential for Ca2+ uptake by ER in neurons and cardiomyocytes[20] (see below). Kv1.3 is present in the PM, in mitochondrial IMM[12], in the cis-Golgi compartment[21] and in the nuclear membrane system[22] (see below). BKCa is present in the PM, Golgi, ER and mitochondria[23]. The role of these channels in the different membranes is only partially established as is the targeting mechanism(s) allowing their localization to different subcellular compartments. The outstanding question is whether the same protein is sorted to different compartments within the cells or specific mechanisms account for their localizations. As for mitoBKCa, a recent study found that this channel in the heart is encoded by a splice variant of the PM BKCa (KCNA1-encoded) and harbors a 50-aa splice insert that is essential for trafficking to the mitochondria[24]. Instead, the proposed mitoKATP component, ROMK2, is a short form of ROMK. To our knowledge, targeting mechanisms have not been identified for the other mitochondrial K+ channels, but interestingly, a short sorting signal in the C-terminal transmembrane domain has been reported to determine targeting of a viral potassium channel to mitochondria versus PM when expressed in mammalian cells[25]. It is important to underline that not all these intracellular channels have been recorded in all tissues, but most of their PM counterparts have relatively wide
been performed on these animals and in TASK-3 KO mice[30] to assess relative importance of these channels (which are often co-expressed in both the PM and intracellular forms of the channels and are prone to mitochondrial function. In summary, the available genetic models target of Kv1.3[28]. ROMK-KO mice have been obtained, although they are other Kv channels has been shown to largely compensate for the lack of compensation [27]. Similarly, in animals lacking Kv1.3, expression of these channels in fine-tuning the oxidative state of the cell [26]. Despite the wide expression and important physiological functions of IKCA, the absence of this channel protein in transgenic KO mice does not result in severe physiological changes, possibly because of developmental compensation [27]. Similarly, in animals lacking Kv1.3, expression of other Kv channels has been shown to largely compensate for the lack of Kv1.3 [28]. ROMK-KO mice have been obtained, although they are short-lived [29]. To the best of our knowledge, no studies have yet been performed on these animals and in TASK-3 KO mice [30] to assess mitochondrial function. In summary, the available genetic models target both the PM and intracellular forms of the channels and are prone to developmental compensation. The generation of adequate cellular/animal models would allow genetic evidence to be obtained regarding the relative importance of these channels (which are often co-expressed in the same tissues) in the regulation of mitochondrial bioenergetics and of the physiological consequences. Nevertheless, numerous studies applying pharmacological agents point to a crucial role of IMM K⁺ channels in the context of energy conversion and cellular protection and provide evidence that K⁺ transport modulates the tightness of coupling between mitochondrial respiration and ATP synthesis. Thus, IMM K⁺ channels contribute to the regulation of matrix volume, in addition to influencing ΔΨmem and ΔpH, calcium transport, production of reactive oxygen species and mitochondrial dynamics. Activation of a mitochondrial calcium-dependent K⁺ channel has been proposed to modulate K⁺ uptake and matrix volume while maintaining mitochondrial membrane potential (ΔΨmem) and to confer protection without compromising oxidative phosphorylation during recovery from metabolic stress [31]. In this case, activation of the channel is proposed to increase bioenergetic efficiency. Other studies instead indicate that a protective mechanism involving the activation of different IMM K⁺ channels includes a slight uncoupling effect, i.e., a slight depolarization leading to increased respiration not coupled to ATP production. Such uncoupling would decrease energetic efficiency [32]. Similarly, activity of the evolutionarily conserved ATP-dependent K⁺ channel mitoKATP has been linked to ischemic preconditioning, ischemic postconditioning, and cytoprotection in general [33,34], even though in heart mitochondria the increased K⁺ influx associated with mitoKATP opening was able to depolarize the membrane by only few millivolts [35]. In addition, a recent piece of work demonstrated that the Kv7.2–7.5 activator margatoxin depolarized the mitochondrial membrane potential, decreased mitochondrial Ca²⁺ levels and in vivo largely prevented the functional and morphological changes triggered by global ischemia/reperfusion in Langendorff-perfused rat hearts, even though ROS production was increased [19]. The exact basis of K⁺ channel openers’ (KCO) cytoprotective properties still remains to be elucidated, although it is suggested that 1) attenuation of ROS production in mitochondria may play a significant role; 2) activation of mitoK⁺ channels controls matrix volume, preserving a narrow intermembrane space, necessary for efficient oxidative phosphorylation; and 3) opening of mitoK⁺ channels produces a mild decrease of membrane potential, thus reducing uptake of Ca²⁺ into the mitochondrial matrix, leading to avoidance of Ca²⁺ overload and subsequent permeability transition pore opening. As mentioned above, most of these studies employed isolated mitochondria and/or relied on the use of non-specific inhibitor or activator drugs displaying pleiotropic effects. The second consideration is especially true for the mitochondrial KATP channel: the bulk of the evidence supporting the involvement of this channel in protection against ischemic/reperfusion damage is pharmacological [36] (see also review by Szewczyk and colleagues, this issue). Unfortunately, other cellular targets possibly accounting for the observed effects have been identified and most, if not all, of the pharmacological agents reportedly activating mitochondrial KATP can behave as membrane-permeable weak acid/base pairs, and thus as uncoupling, ΔΨmem-dissipating agents, possibly accounting for their protective effects. But specificity issues apply also to the other KCOs. For example, the CGS7184(ethyl1-][(4-chlorophenyl)amino]oxo]-2-hydroxy-6-trifluoromethyl-1H-indole-3-carboxylate, a synthetic BKCa channel opener, directly activates the ryanodine receptor calcium release (RyR2) channel in the sarcoplasmic reticulum [37].

Besides a role in cytoprotection, modulation of mitochondrial K⁺ channels can also lead to cell death and to regulation of autophagy. Recently it has been reported that in vascular smooth cells Angiotensin II increased ROS production and autophagy while inhibitors of mitochondrial KATP channels contrasted both events [38]. The example studied in most detail in the context of cell death is the mitochondrial Kv1.3 channel. MitoKv1.3 mediates an inward potassium flux to the mitochondrial matrix and likely has a role in the organellar K⁺ cycle that participates in the modulation of coupling between ATP synthesis and mitochondrial respiration [39]. In vivo evidence has been obtained suggesting that modulation of mitoKv1.3 by pharmacological means represents an unconventional but promising strategy to selectively eliminate cancer cells. Kv1.3 is overexpressed in various cancer tissues/cells and expression of PM-located Kv1.3 seems to correlate with that of the mitochondrial counterpart (mitoKv1.3). MitoKv1.3 was identified as a novel target of Bax: physical interaction between the two proteins via K128 of Bax took place in apoptotic cells, leading to inhibition of channel activity [40,41] and consequent ΔΨmem changes, increased ROS production and cytochrome c release, whereas Kv1.3-deficient mitochondria were resistant. In agreement with these results, Psora-4, PAP-1 and clofazimine, three distinct membrane-permeant inhibitors of Kv1.3 [42], induced death in multiple human and mouse cancer cell lines by triggering the same series of events. In contrast, membrane-impermeant, selective and high-affinity Kv1.3 inhibitors ShK or Margatoxin did not trigger apoptosis, suggesting a crucial role for the mitochondrial Kv1.3 versus PM Kv1.3. Genetic deficiency or siRNA-mediated downregulation of Kv1.3 abrogated the effects of the drugs, proving specificity of their action via Kv1.3. In a preclinical mouse model, intraperitoneal injection of clofazimine significantly reduced melanoma tumor size while no adverse effects were observed in several healthy tissues [43]. Furthermore, these drugs induced death of only the pathological primary tumor cells from B-cell chronic lymphocytic leukemia patients [44]. As to other IMM K⁺ channels, inhibition of IKCA by TRAM-34 increased the sensitivity of melanoma cells to TRAIL treatment [45]. Mitochondrial TASK-3 channels are also likely to contribute to the regulation of apoptosis since their silencing resulted in compromised mitochondrial function, i.e. mitochondrial membrane depolarization, and reduced cell survival inducing apoptotic cell death in melanoma cells [46]. Recently a modified channel function of mitochondrial BKCa has been linked to amyloid-beta (Aβ)-induced neuronal toxicity [47]. In summary, as a result of the above difficulties in the field, the relationship between mitochondrial ion transport and diseases linked to altered mitochondrial function is still only partially explored, but the so-far available data point to IMM K⁺ channels as possible targets for therapeutic application against various pathologies.
3. Mitochondrial potassium channels in plants

Mitochondria in plants are not only the site of respiration and metabolic processes typical of animals, but are in charge of a specialized plant-specific process, the photosrespiration [48]. This process serves to recycle metabolic products formed during the assimilation phase, due to the oxygen-fixing ability of the carbon-fixing enzyme Rubisco, and involves chloroplasts, peroxisomes mitochondria and the cytosol. In particular, O₂ fixation by Rubisco leads to the generation of one molecule 3-phosphoglycerate and of one molecule 2-phosphoglycolate. This latter metabolite inhibits important enzymes, thus must be detoxified via photorespiration which yields CO₂ [49]. Thus, in plants, mitochondria, by impacting on photorespiration, may alter photosynthetic efficiency as well. Several ion channel activities have been recorded in plant mitochondria, mostly using the electrophysiological planar lipid bilayer technique and isolated membrane vesicles (for recent review see [50]). Among potassium channels, an ATP-regulated potassium channel [pmitoKATP channel], a large-conductance Ca²⁺-insensitive iberiotoxin-sensitive potassium channel, and a large conductance, calcium-activated, iberiotoxin-sensitive potassium channel (pBKCa) have been identified [51,52]. Classical bioenergetics studies identified also other mitochondrial potassium channels, including a cyclosporine A-activated and ATP-inhibited mitochondrial potassium channel [53] and a quinine-inhibited but ATP-insensitive potassium channel [54]. Characteristics of the pmitoKATP have been investigated in depth also using classical bioenergetics (e.g. [55,56], for recent review see [57]). Similarly to the animal mitoKATP, the plant channel has also been shown to affect ΔΨm and ROS production. According to the current view, when ATP inhibits pmitoKATP, ΔΨm remains low (hyperpolarized) and ROS production is high. PmitoKATP would act as one of the dissipative systems that may allow influx of positive charges into the matrix and thus partially depolarize ΔΨm in order to avoid excessive ROS production under environmental stresses. Indeed, determination of the in vivo dynamics of the membrane potential in individual mitochondria in plants has demonstrated that these organelles undergo rapid and reversible partial dissipation and restoration of ΔΨm [58]. A similar situation has been described for pBKCa, i.e. activation of the channel by Ca²⁺ and NS1619 stimulated resting respiratory rate and caused partial mitochondrial membrane depolarization, while the opposite effects could be observed upon inhibition by iberiotoxin [51].

In summary, the original hypothesis [59,60] which predicts that dissipative pathways, including opening of mitochondrial potassium channels in the IMM leading to a slight depolarization, would cause mild uncoupling and a consequent reduction in further mitochondrial ROS generation according to a feedback mechanism, seems to apply to both the animal and plant systems, although to different extents depending on the examined species. It also has to be stressed that the genetic evidence obtained so far is not sufficient to prove or negate this idea either in plants or in animals.

4. Chloroplast potassium channels

Several potassium-selective ion channels with different conductance and characteristics have been recorded from the outer envelope, inner envelope and thylakoid membranes of chloroplasts as summarized in comprehensive and thorough reviews (e.g. [50,61–64]). Unfortunately, pharmacological characterization of these channels is rather limited. In addition, patch clamping chloroplasts from the completely sequenced Arabidopsis model plants represent a so-far unsurmounted technical difficulty. Therefore, the proteins giving rise to most activities are undefined. Nonetheless, an important function has been proposed in the regulation of stroma alkalinization via counterbalance of H⁺ movement across the inner envelope membrane, especially for an ATP-sensitive potassium channel [65] and for the fast-activating cation channel of the envelope membrane [66]. During illumination, due to H⁺ pumping by the ATPase of the inner envelope membrane, the membrane potential across this membrane reaches ~100 mV (inside negative). This H⁺ extrusion is supposed to be balanced by K⁺ influx across the cation selective channels mentioned above.

Such a counterbalancing role has been hypothesized [67] also for the cation channels detected by electrophysiological studies in the thylakoid membrane [68–71]. Like respiration, photosynthesis leads to the generation of a proton motive force, composed of a proton gradient (the ΔpH) and of an electric field (the ΔΨ). Generation of ΔpH is the result of chloroplast lumen acidification following plastoquinol (PQH₂) oxidation by the cytochrome b⁶f complex and water oxidation by Photosystem II (PSII), ΔΨ is instead the result of charge separation in PSII and PSI and of the activity of the cytochrome b⁶f complex [72]. The ATP synthase–ATPase CF₀–F₁ complex translocates H⁺ and thus modifies the ΔΨ and ΔpH at the same time, but cannot change the relative composition of the pmf [73]. Instead, ion channels are expected to modify the ΔΨ/ΔpH ratio, by varying the electric field without directly affecting the proton gradient. Thus, a partial dissipation of ΔΨ either by cation efflux from or by anion influx into the thylakoid lumen might ensure further entry of the positively charged protons therefore contributing to the establishment of ΔpH. Importantly, the photoprotective mechanism known as non-photochemical quenching (NPQ) which is crucial for dissipation of excess absorbed light, is triggered by ΔpH (acidic pH in the lumen) and ΔpH modulates also the rate of electron transfer.

The first genetic proof in favor of the counterbalance hypothesis has been obtained in cyanobacteria, in which the voltage-gated potassium channel SynK was found to be located in the thylakoid membrane [74]. Its lack in knock-out organisms was shown to affect ΔpH, ultimately leading to decreased growth under high light culturing conditions [75]. Later, the two-pore potassium channel TPK3 was reported to play a similar role: silenced plants lacking AtTPK3 grown at a light intensity that was fully tolerated by WT plants, exhibited a decreased rosette size and an increased light sensitivity as assessed by measuring photosynthetic parameters and anthocyan content. TPK3 is not voltage-gated but it becomes activated by increasing calcium concentration and acidic pH. In the case of TKP3, using Arabidopsis plants, it was possible to evaluate the pmf partitioning using the electrochromic shift methodology which is based on the observation that ΔΨ induces a shift in the absorption spectra of some photosynthetic pigments [76,77]. Thus, the phenotype reflected the observed altered pmf partitioning, i.e. higher ΔΨ and lower ΔpH due to the lack of counterbalancing flux of positively charged potassium ions. This, in turn resulted in reduced CO2 assimilation, reduced growth and also in a deficient NPQ [78]. In contrast, the KEA3 K⁺/H⁺ exchanger was shown to balance the ΔpH and ΔΨ during transient light shifts and in the dark, when the K⁺ and H⁺ gradients return to the situation preceding illumination [79,80]. Overall, these studies have opened a novel, fast-developing field of investigation: the fine-tuning of photosynthesis by ion homeostasis [81]. To complete these studies several questions have to be answered, first of all regarding the identification of further ion channels mediating the fluxes of anions and divergent cations in the thylakoid membrane.

5. Nuclear potassium channels in animals

Although various ion channels are present and functional in the outer and inner nuclear envelope membranes as assessed by biochemical and electrophysiological methodologies (for review see e.g. [82, 83]), surprisingly little is known about their function. The outer nuclear membrane is continuous with the endoplasmic reticulum so that the perinuclear space of the nuclear envelope is contiguous with the lumen of the endoplasmic reticulum. The perinuclear space (nuclear envelope lumen) is assumed to be the Ca²⁺ store, generating a concentration gradient across the inner nuclear envelope with high Ca²⁺ levels in the nuclear envelope lumen and low Ca²⁺ levels outside (i.e. in the nucleoplasm and in the cytosol). The ion gradient for K⁺ across the nuclear envelope is not known in intact cells, but studies in isolated nuclei indicate that the K⁺ concentration in the perinuclear space is
much lower than in the cytoplasm and nucleoplasm [84]. Therefore, it is expected that the changes in the nuclear K⁺ channel activity would alter the flux of this cation, leading to an alteration of the nuclear ΔΨ. The electrical potential difference across the nuclear envelope is the result of various factors comprising intranuclear electrical charges, diffusion of ions across the inner and outer nuclear membranes and diffusion along the nuclear pore complex and may reach −30 mV (negative inside the nucleus) [83].

More than ten years ago a seminal paper demonstrated the presence of a functional KATP channel in the nuclei of beta pancreatic cells and linked glucose metabolism to nuclear Ca²⁺ signals and nuclear function. In particular, the authors provided evidence that pharmacological blockade of the KATP channel in isolated nuclei triggered nuclear Ca²⁺ transients and induced phosphorylation of the transcription factor cyclic AMP response element binding protein CREB. Indeed, as was shown for the first time in hippocampal neurons, signaling to CREB can be activated by nuclear calcium alone and does not require import of cytoplasmic proteins into the nucleus [85]. In intact pancreatic cells, fluorescence in situ hybridization revealed also that these Ca²⁺ signals were able to elicit c-myec expression [86]. A similar role has been envisioned for the BKCa channels, detected in the nuclear envelope of rodent hippocampal neurons: their blockade was shown to induce nuclear–derived Ca²⁺ release, nucleoplasmic Ca²⁺ elevation and CREB–dependent transactivation and to regulate nuclear Ca²⁺–sensitive gene expression. To our knowledge, this important work established for the first time a link between a nuclear potassium channel and neuronal activity, also using a genetic model (Kcnma1−/− mice) [87]. Recently, evidence indicates that a shaker-like Kv channel, Kv1.3, has also a nuclear localization besides being active in the PM and in mitochondria (see above). Kv1.3 channels were found to be expressed in the nuclei of various cancer cells but also of normal human brain tissues [22]. The authors reported that the selective Kv1.3 blocker Margatoxin was able to induce hyperpolarization of the nuclear membrane in isolated nuclei suggesting functional expression. Furthermore, addition of a membrane-permeant Kv1.3 inhibitor, PAP-1, to intact lung adenocarcinoma cells resulted in an increased phosphorylation of CREB and of c-Fos, an immediate early response transcription factor. The channel was also shown to form a complex with the upstream binding factor 1 in the nucleus. Interestingly, these authors reported that other Kv channels, namely Kv1.1, Kv1.2 and Kv2.2 displayed either prevalent nuclear or mainly PM localization depending on the cancer cell line used for their studies [22].

Other reports also point to a nuclear localization of channels that are normally known to be expressed in the PM. For example, immunohistochemical staining of endobronchial biopsies from healthy subjects revealed that KCa3.1 channels are localized in the plasma membrane and nucleus of airway smooth muscle cells. However, it is still unclear whether it is the nuclear or the PM channel whose inhibition by membrane permeant inhibitor TRAM-34 causes enhanced corticosteroid activity in severe asthma [88]. In another study the inward rectifying Kir2.2 was found in the nucleus in sections of rat hindbrain and dorsal root ganglia tissue, but again, the functional importance of this localization is unknown [89].

A few years ago, a surprising localization of the human ether α-go-go1 protein (Eag1 or Kv10.1), has been described in the inner nuclear membrane in both human and rat models [90,91]. Kv10.1 is a member of the voltage-gated potassium channels (subfamily H) and its peculiarity is that it was the first ion channel demonstrated to have oncogenic properties, since transfection of Kv10.1 into mammalian cells conferred a transformed phenotype and favored tumor progression in vivo [92,93]. Kv10.1 is not detected in healthy tissues except the brain, but its overexpression has been detected with a very high incidence (>75%) in several human malignancies, including glioblastoma, colorectal and cervical cancer, soft tissue sarcomas, acute myeloid leukemia, esophagus and gastric cancer [94,95]. High Kv10.1 expression is associated with poor prognosis in fibrosarcoma, ovarian carcinoma, acute myeloid leukemia (AML), in colon, head and neck cancer [94]. An increased expression of Kv10.1 is correlated with downregulation of microRNA miR-296-3p as observed in glioblastoma [96] and is controlled also by the p53 tumor suppressor-miR-34-E2F1 transcription factor pathway [97]. Overexpression of Kv10.1 promotes cancer cell migration and proliferation by interactions with various proteins, including cortactin and focal adhesion kinase (FAK), as well as through regulation of calcium signaling [94].

Interestingly, Kv10.1 channels were shown to regulate intracellular signaling pathways independently of their ability to mediate ion flux [98,99] and a mutation that abolished potassium permeability did not prevent tumor formation by transfected cells in vivo [100], suggesting that channel function is not mandatory for the oncogenic potential of Kv10.1. These findings are in line with the observations that the majority of Kv10.1 protein remains in intracellular pools, including the perinuclear region. Interestingly, even if expressed in the plasma membrane, Kv10.1 becomes rapidly (within 30 min) internalized and localizes to the inner nuclear membrane where it is functionally active as assessed using patch clamping. A conserved nuclear localization signal is present on the C-terminal cytoplasmic domain of Kv10.1, but this signal does not seem to be required for this localization, which may contribute to the oncogenic properties of the channel by a still unknown mechanism [101]. The authors hypothesized that the channel may indirectly interact with heterochromatin or may influence gene expression and genome stability by changing K⁺ concentration, known to affect the stability of transcriptional repressor elements (for example of the myc oncogene) [102].

In summary, several K⁺ channels, known to reside in the PM, can reach the nuclear envelope and exert their function there. Most likely these channel proteins do not follow a route to the nucleus via ER but via recycling from the PM [101]. This nuclear localization does not seem to be correlated with pathological conditions; on the contrary, even in healthy cells it links nuclear membrane potential changes, alteration of nuclear calcium concentration and activation of transcription factors ultimately leading to changes in gene expression.

6. Nuclear potassium channels in plants

In animal cells, Ca²⁺–permeable channels such as ryanodine receptors and inositol triphosphate receptors (InsP3R) localized in the nuclear envelope or ER are involved in the generation of Ca²⁺ oscillations in the perinuclear region [103]. In plants, in particular in Lotus japonicus and Medicago truncatula, perinuclear calcium spiking in leaf root symbiosis was shown to require two potassium-permeable channels, Castor and Pollux in Lotus [104] or their homolog, Does Not Make Infections 1 (DMI1) in Medicago [105,106]. Interestingly, Castor and Pollux have been identified through a screening aiming at identifying genes involved in the transcriptional reprogramming of the root during symbiosis [107]. The selectivity filter of Castor and Pollux contains the sequence ADSGNHA instead of the classical TVGYGD sequence, while the M. truncatula Pollux ortholog DMI1 contains ADAGNHA. Castor and Pollux are not potassium-selective channels, and show only a moderate preference for K⁺ over Na⁺ and Ca²⁺. Evidence has been gained that Castor and Pollux (and DMI1 alone in Medicago) modulate the nuclear envelope membrane potential and thereby either trigger opening of nuclear calcium release channels and/or compensate the charge release during the calcium efflux as counter ion channels. In particular, the Parniske group suggested the following model: DMI1 or Castor and Pollux together would be required to mediate the flux of K⁺ ions along their concentration gradient from the cytoplasm to the perinuclear space. This sustained flow would lead to subsequent rapid hyperpolarization of both nuclear membranes which is required to activate the nuclear Ca²⁺ channel. Ca²⁺ then flows out of the perinuclear space to the cytoplasm, giving rise to a Ca²⁺ spike. The DMI1 and Castor/Pollux cation channels, upon sensing the Ca²⁺, would be blocked or inactivated. Simultaneously, the Ca²⁺ flow would reduce the
hyperpolarization of the nuclear membranes, resulting in the closure of hyperpolarization-activated Ca\(^{2+}\) channels. Finally, the Ca\(^{2+}\) ions would be pumped back into the perinuclear space (the calcium store), by the Ca\(^{2+}\) ATPase bringing the membrane back to its resting potential, ready to reinitiate the cycle. In summary, these plant nuclear channels would act as counter-ion channels that compensate for the positive charge associated with Ca\(^{2+}\) release during perinuclear calcium spiking [108].

7. SR/ER/golgi-located and lysosomal potassium channels

Ca\(^{2+}\) release from the intracellular stores sarco/endoplasmic reticulum (SR/ER) crucially regulates cellular functions including muscle contraction, gene transcription, secretion and cellular metabolism. When Ca\(^{2+}\) is released from the SR/ER, a negative potential would be generated on the SR/ER luminal side as a result, and this would be expected to inhibit subsequent Ca\(^{2+}\) release. On the other hand, the generation of a positive potential within the SR/ER lumen during Ca\(^{2+}\) uptake would tend to inhibit Ca\(^{2+}\) pumping function. It is therefore likely that potent counter-ion movements (for example via potassium channels) are essential to balance the SR/ER membrane potential and maintain efficient Ca\(^{2+}\) uptake/release from/to this intracellular calcium store [109,110]. The role of functional potassium channels in the Golgi (e.g. Kv1.3 [21]) is less intuitive.

Numerous potassium channels have been described in the endo/sarcoplasmic reticulum (for recent review see [111]). Among these, KATP [112], SKCa [20] and the voltage-gated K\(^+\) channel Kv1.6 [21] are likely counterparts of the PM channels, while the trimeric intracellular cation (TRIC) channels [113] which are also permeable to potassium, represent a specific intracellular form. For these latter channels it has been clearly demonstrated in an elegant piece of work that TRIC is required for K\(^+\) permeability of the skeletal muscle sarcoplasmic reticulum, as well as for correct Ca\(^{2+}\) signaling. TRIC-knockout mice suffered from embryonic cardiac failure and mutant cardiac myocytes showed severe dysfunction in intracellular Ca\(^{2+}\) handling. Thus, this work provided compelling evidence that TRIC channels are likely to act as counter-ion channels that function in synchronization with Ca\(^{2+}\) release from intracellular stores [113]. A similar conclusion has been reached in neurons and cardiomyocytes in another investigation, whose authors reported that inhibitors of SKCa channels blocked the uptake of Ca\(^{2+}\) by the ER, whereas inhibitors of IKCa, BKCa and KATP had no effect [20]. What is the relative contribution of TRIC and SKCa in cardiomyocytes to charge counterbalancing and whether one of the two channels may prevail depending on different (patho)physiological conditions, are still open questions.

In plants, to our knowledge, no potassium channel with such a function has been identified. AtKCI, a silent Arabidopsis potassium channel alpha subunit, which is reportedly not able to form functional ion channel on its own [114], was shown to reside in the ER unless it is co-expressed with other shaker-type inward rectifier subunits, such as AKT1, KAT1, KAT2 and AKT2 [115]. Only in this case, the shaker alpha subunit-AtKCI tetramers, displaying different biophysical properties with respect to AKT1 or KAT1, KAT2 and AKT2 homotetramers alone, relocate to the plasma membrane [115,116]. Whether the tetramers formed in the ER are functional and whether they may contribute to counterbalance during ER calcium release/signaling in plants is still unexplored.

Just a few months ago, the first protein underlying the lysosomal/endosomal potassium conductance has been identified [117]: in this seminal paper, the authors discovered that the potassium channel recorded by patch clamp directly on the organelle is formed by TMEM175, a protein with previously unknown function. In the past, the two-pore potassium channel K2P1 protein has been detected in endosomes using immunostaining [118], but the measured lysosomal K\(^+\) current was independent of K2P1 expression [117]. Similarly to Castor, Pollux and DMI1, the protein TMEM175 does not harbor the typical selectivity filter sequence of K\(^+\) channels. Yet, TMEM175 is highly selective for potassium. Lysosomes lacking TMEM175 exhibited no K\(^+\) conductance, had a markedly depolarized \(\Delta\Psi\) and displayed little sensitivity to changes in \(\text{[K}^{+}\])\]. Interestingly, lack of this protein conferred compromised luminal pH stability, indicating a role for potassium as counterion to maintain acidic pH in the lumen. Abnormal fusion with autophagosomes during autophagy also occurred in the KO cells. It is thus highly likely that this channel will join to the list of lysosomal channels whose mutation is linked to severe diseases, including neuronal degeneration and lysosomal storage diseases [119,120].

8. Vacuolar plant potassium channels and peroxisomal cation channels

No osmotic gradient persists across the membrane of plant storage-organelles, the vacuoles, since their membrane is permeable to water. A large amount of inorganic ions acting as osmolytes is accumulated in plant vacuoles via specific channels and transporters [121–123]. An electrochemical gradient favoring the efflux of potassium ions from vacuoles towards the cytosol exists. Several K\(^+\) channels have been shown to reside in the membrane of the large central lytic vacuole or of protein storage vacuoles, all of them belonging to the two-pore potassium TPK channel family which is comprised of 6 members [124–126]. AtTPK4 is targeted to the PM, while the other members localize to the vacuolar membrane (but see above TPK3 in the thylakoid). However, the roles of vacuolar TPKs are still largely unknown [125]. The best characterized TPK is AtTPK1, whose activity is regulated by calcium, by interaction with 14–3–3 proteins, by luminal pH [127] and by membrane tension [128]. Transgenic plants either lacking or overexpressing this channel highlighted that TPK1 has a function in intracellular K\(^+\) homeostasis, affects germination, seedling growth and stomatal movement [129]. TPK channels have been proposed to underlie the so-called vacuolar K\(^+\) (VK) channel which is highly selective for K\(^+\) ions [130,131], is fast-activating and has thus been postulated to play a major role in guard cell turgor regulation and K\(^+\) homeostasis. VK channels were proposed to contribute to a calcium-induced calcium release from the vacuoles, by shifting the resting vacuolar membrane potential (see e.g. [131]).

In the peroxisomal membrane two proteins, namely Pex11 and Pxmmp2, both giving rise to high conductance channels with slight preference for cations, have been characterized. The yeast Pex11 pore-forming protein shares sequence similarity with transient receptor potential melastatin (TRPM) cation-selective channels and forms a voltage-independent channel with a conductance of 4.1 nS in 1.0 M KCl [132]. The other weakly cation selective channels are formed by Pxmmp2 and display a conductance of 1.3 nS in 1.0 M KCl [133]. Both proteins have been proposed to mediate the flux of small metabolites across the peroxisomal membrane.

9. Conclusion

In summary, the number of potassium channel proteins located in intracellular membranes is rapidly increasing. A part of these proteins displays a classical selectivity filter sequence in their pore loop, while novel, unexpected proteins without this signature sequence have also been identified and shown to function as potassium channels. This finding suggests that in the future, other, atypical proteins might emerge as potassium channels. The major part of the identified channels are counterparts of the PM-located well-known K\(^+\) channels, and some of them show even multiple localizations within the cells, while other channels are exclusively located in organelle membranes. Several open questions will have to be addressed in future studies related to e.g. targeting and molecular identity, in order to fully appreciate the physiological functions of these ion channels. In addition to ion channels, of course a plethora of potassium-transporting carriers have been described and studied.
in the various membranes, whose function is likely to be well-coordinated with that of the channels.

Transparency document

The Transparency document associated with this article can be found, in online version.

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