The secret life of ion channels: Kv1.3 potassium channels and proliferation

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Abreviations:

4-aminopyridine ***(4-AP)***; Antigen presenting cells ***(APCs)***; Calcium Release-Activated Ca^{2+} channels ***(CRAC)***; Central memory T-cell ***(T_{CM})***; Effector memory T-cell ***(T_{EM})***; Extracellular matrix ***(ECM)***; Intermediate conductance calcium activated K^+ channels (***KCa3.1 channels)***; Inwardly rectifier K^+ currents ***(K_{IR})***; Lipopolysaccharide ***(LPS)***; Membrane potential ***(V_M)***; outwardly delayed rectifier K^+currents ***(K_{DR})***; Oligodendrocyte progenitor cells ***(OPCs)***; phenotypic modulation ***(PM)***; Tyrosine kinases ***(TK)***; Vascular smooth muscle cells ***(VSMCs)***; Voltage-dependent potassium channels ***(Kv channels)***.

Abstract

Kv1.3 channels are involved in the switch to proliferation of normally quiescent cells, being implicated in the control of cell cycle in many different cell types and in many different ways. They modulate membrane potential controlling K^+ fluxes, sense changes in potential and interact with many signaling molecules through their intracellular domains. From a mechanistic point of view, we can describe the role of Kv1.3 channels in proliferation with at least three different models. In the "*membrane potential model*", membrane hyperpolarization resulting from Kv1.3 activation provides the driving force for Ca^{2+} influx required to activate Ca^{2+}-dependent transcription. This model explains most of the data obtained from several cells from the immune system. In the *"voltage sensor model"* Kv1.3 channels serve mainly as sensors that transduce electrical signals into biochemical cascades, independently of their effect on membrane potential. Kv1.3-dependent proliferation of vascular smooth muscle cells (VSMCs) could fit this model. Finally, in the *"channelosome balance model"*, the master switch determining proliferation may be related to the control of the Kv1.3 to Kv1.5 ratio, as described in glial cells and also in VSMCs. Since the three mechanisms cannot function independently, these models are obviously not exclusive. Nevertheless, they could be exploited differentially in different cells and tissues. This large functional flexibility of Kv1.3 channels surely gives a new perspective on their functions beyond their elementary role as ion channels, although a conclusive picture of the mechanisms involved in Kv1.3 signaling to proliferation is yet to be reached.

Introduction

The early studies linking K^+ channels to cell proliferation were carried on T lymphocytes in the early 80´s (49). Since then, K^+ channels have been identified as important contributors to cell proliferation both in normal and cancerous cells (158, 170). K^+ channels are important for setting the resting membrane potential (V_M) and the driving force for Ca^{2+} influx, and both mechanisms contribute in many different ways to cell cycle progression (118). In addition, both are decisive for volume regulation of growing cells (92). Finally, it is increasingly recognized that K^+ channels can control cell proliferation through other signaling mechanisms independent of ion fluxes (71, 80). Their functional flexibility together with their location at the plasma membrane makes them suitable for coordinating multiple signaling pathways, integrating intra and extracellular signals.

A wide variety of K^+ channels has been shown to contribute to the regulation of different cell cycle checkpoints in different cancer cells (90, 93, 118, 126). These data rely on the observation that genetic or pharmacological blockade of K^+ channels inhibit cancer cells growth. Although cancer and normal cells can share some mechanisms, the aberrant expression, modulation and/or localization of K^+ channels in cancer cells have been shown to alter the downstream signals that converge on the cell cycle machinery. In this review, we will focus on what is known regarding the mechanisms by which a voltage dependent K^+ channel, the Kv1.3, modulate cell proliferation under physiological or pathological conditions in non-cancerous cells.

Interestingly, in three tissues in which a role for Kv1.3 channels in proliferation has been determined, different mechanisms have been proposed. ***In the immune system***, and particularly in T-cells, many studies argue in favor of a mechanism of Kv1.3 influence on proliferation involving K^+ efflux and membrane hyperpolarization. By favoring Ca^{2+} influx, this hyperpolarization promotes the activation of Ca^{2+}-dependent transcriptional factors leading to proliferation. This mechanism could be defined as the ***"membrane potential model".*** In contrast, in ***glial cells of the nervous system***, proliferation signals conveyed by Kv1.3 channels are related to changes in the balance of expression of several Kv channels, most notably Kv1.5 and Kv1.3. This model could be designated as the "***channelosome balance model"***. Finally, the available data in ***vascular smooth muscle*** share some of the characteristics of this balance model, although data from heterologous expression systems point to the importance of Kv1.3 channels as voltage sensors, since the effect of Kv1.3 on proliferation could be reproduced by non-conducting mutants (40). This illustrates the so called ***"voltage sensor model"***. Clearly, these mechanisms are not exclusive, but from a didactical point of view it could be useful to dissect them independently.

An overview of Kv1.3 channels

Kv1.3 (KCNA3) is a voltage-gated K^+ channel belonging to the shaker related (Kv1, KCNA) subfamily. The existence of voltage-dependent potassium (Kv) channels was first reported in excitable cells, although they are almost ubiquitously expressed. In 1984, a delayed-rectifier Kv

channel showing similarities with their counterparts in nerve and muscle was described in human T-cells (50) and classified as the n-type channel, for its prevalence in "normal" human T-cells (51, 99). After the finding of the *Drosophila* mutant *Shaker,* the cloning of a family of K^+ channels from the *Shaker* locus (81, 125, 134, 142) provided the tools to identify Kv channels. Using low stringency hybridization protocols Kv1.3 channel was isolated from mouse and rat brain (30, 64, 151, 152) in different laboratories and with different names. The adoption of a consensus nomenclature for Kv channels solved this confusion (28, 67).

Kv1.3 structure

Kv channels are tetramers of pore-forming α subunits, each one with six transmembrane helices (S1-S6) connected by intra- and extracellular loops (Figure 1A). The N- and C-terminal domains are intracellular regions of variable length. The basic structural elements have been confirmed from the crystal structure of their bacterial homologues KcsA and KaVP (52, 77, 102).

The transmembrane helices of each Kvα subunit contain two functionally and structurally independent domains: The K^+ selective pore (formed by S5 and S6 helices and their linker) and the voltage sensor domain (the S1-S4 transmembrane helices). The pore has a motif of five amino acids (TVGYG) forming the narrowest part of the channel (the selectivity filter) which is highly conserved among K^+ channels. The four pore domains arrange symmetrically around the conduction pathway, surrounded by the four voltage sensor domains. The S4 segment contains a positively charged amino acid every third position and accounts for most of voltage dependence of the channel gating. Membrane depolarization induces a structural rearrangement of S4 which translates into a displacement of the S4-S5 linker that opens or closes the conduction pathway (17, 77, 127).

The cytoplasmic domains participate in channel assembly and association with auxiliary subunits as well as in the regulation of inactivation and trafficking. Tetramerization of Kvα subunits is subfamily-specific and involves a highly conserved N-terminal domain (the T1 domain). Also, Kvα subunits can assemble with ancillary Kvβ subunits through this T1 domain, increasing functional heterogeneity (101, 143). More recently, a growing number of studies indicate that these cytoplasmic domains can have roles beyond regulation of K^+ fluxes, participating in cell signaling via protein-protein interactions (8, 95). The large divergence in the sequence of these regions within the same family suits with the subunit-specific nature of these interactions.

Kv1.3 biophysics

Members of the Kv can be identified based on their specific biophysical and pharmacological properties. These properties are better characterized in isolation, by using heterologous expression systems. The correlation of these parameters with native currents may be poor due to the presence of a mixture of homo- and heterotetrameric channels, to their association with accessory subunits and to post-translational modifications (reviewed in 36). In the case of Kv1.3 channels the biophysical properties of the heterologously expressed channels are very similar

to the endogenous Kv1.3 currents observed in T-cells, as Kv1.3 is the only Kv subunit expressed.

Kv1.3 activation and inactivation is voltage-dependent. Activation threshold occurs at potentials between -60 and -50 mV. Open probability has a very steep voltage-dependence, with a midpoint around -40 mV (reviewed in 106). The voltage-dependence of inactivation is also very steep, with a midpoint around -45 mV for heterologous channels and between -60 and -70 mV for native T-cell currents, due to the modulatory effect of accessory Kvβ2 subunits (1, 109). These biophysical properties determine a small range of voltages at which there is a finite probability that Kv1.3 channels are open and not inactivated (i.e. the window current region). In T-cells, this region overlaps with resting V_M, allowing its effective control by Kv1.3 currents (86, 120).

Kv1.3 activation time course is fast (a few milliseconds) whereas the inactivation time constant is much larger, ~200 ms at +50 mV (122). Kv1.3 channels inactivate with the slow C-type inactivation, caused by the rearrangement of the outer mouth of the permeation pathway (38, 76, 103, 138). C-type inactivation is sensitive to extracellular medium composition: it is slowed by elevated extracellular K^+, and, in the case of Kv1.3, is also slowed at acidic pH_e (98, 147). These changes are profound, and Kv1.3 channels require a considerable time to reopen again, exhibiting a remarkable cumulative inactivation (100, 123, 138). Many of these biophysical and kinetic properties are relevant for the modulation of firing patterns in excitable cells. However, their possible role shaping membrane potential in non excitable cells remains to be elucidated. In any case, it is clear that due to these distinct properties, replacement of Kv1.3 channels for other Kv1 channels will impact resting V_M also in non excitable cells (see below).

Kv1.3 pharmacology

Molecules inhibiting Kv1.3 channel fall into two structural categories: venom-derived peptides, and organic small molecules. They can affect Kv1.3 currents by blocking the ion-conducting pore from the external or internal side or by modulating channel gating through binding to the voltage-sensor domain or auxiliary subunits. The number of selective and specific Kv1.3 blockers is continually growing, due to their relevance in T-cell physiology.

Initially, Kv1.3 blockers were viewed as general immunosuppressants assuming that they inhibited calcium influx in all T-cell subsets. They were proposed as alternatives to cyclosporine and FK506 for prevention of transplant rejection. However, the discovery of a prominent role for Kv1.3 channels in a T-cell subset (called effector memory T-cell or T_{EM}) involved in the pathogenesis of autoimmune diseases (171) changed the concept and the strategy. Now Kv1.3 blockers are regarded as immunomodulators that can selectively suppress human T_{EM} (173).

Many peptides obtained from scorpion venom such as charybdotoxin, margatoxin, kaliotoxin, agitoxin-2 and noxiustoxin can block Kv1.3 channels. They are peptides of 18-60 residues cross-linked by 2-4 disulfide bridges, forming molecules remarkably resistant to denaturation. Recently, another very potent Kv1.3 blocker, ShK, was isolated from the Sea anemone

Stichodactyla helianthus (27). Extensive work has focused on the development of more stable and specific Shk analogous for the treatment of autoimmune diseases (13, 29, 130, 156). These peptides bind tightly into the external vestibule of the channels with 1:1 toxin-channel stoichiometry, occluding the permeation pathway (review in 107, 151). Altogether, they constitute a large pharmacological armamentarium to target Kv channels with high potency and specificity (173).

The first small molecules that were used to pharmacologically identify K^+ channels were the classic Kv channel inhibitors 4-aminopyridine (4-AP) and TEA, and the Ca^{2+} activated K^+ channel blocker quinine (173). They are organic cations that block open K^+ channels by binding to the inner pore from the cytoplasmic side. After Kv1.3 cloning, a high-throughput screening of library compounds was used to search for more potent and selective small molecule blockers with little success. Meanwhile, in the early 90´s several reports suggested that extracts of *Ruta graveolens* alleviated multiple sclerosis symptoms, an effect related with their blocking effect on K^+ channels (21). 5-methoxypsoralen (5-MOP), a compound used for psoriasis treatment, was identified as the major K^+ channel channel-blocking principle of *R. graveolens*. Derivatives without photoreactivity, and with higher potency and selectivity for Kv1.3 channels such as the phenoxyalkoxypsoralen-1 (PAP-1) were developed afterwards (141, 161).

Kv1.3 modulation

Ion channels associate with protein adaptors, kinases, second messenger producing enzymes, membrane receptors and cytoskeletal proteins, forming the so called channelosome. This macromolecular complex enables the spatio-temporal organization of cellular responses and regulates the channel localization, activity and function, converting ion channels in master regulators of functions such as adhesion, migration, and proliferation. Several types of proteins have been described within the Kv1.3 channelosome (Figure 1B).

Auxiliary subunits

Although heterologous expression of Kvα subunit recapitulates voltage-dependent K^+ fluxes, it is now clear that native Kv channels are heteromultimeric complexes coassembled with accessory subunits. These auxiliary subunits have, in addition to their influence on Kv channel kinetics, important roles in Kv channel sorting and trafficking to distinct cellular localizations (135). Kv1.3 associates with Kvβ and KCNEs auxiliary subunits.

Kvβ are cytosolic proteins that bind to T1 cytoplasmic domains of Kvα subunits with a four-fold symmetry so that Kv channels are tightly associated 4α:4β octamers (135). In human T-cells, Kvβ2 subunits accelerate the assembly of Kv1.3 channels, enhancing current levels (109). In addition to Kvβ, KCNE peptides, which have been widely studied in association with Kv7 and Kv11 cardiac channels, also contribute to Kv1.3 regulation. KCNE4 behaves as a dominant-negative partner of Kv1.3 (65), leading to decreased current density, slower activation and accelerated inactivation(146). Moreover, by masking a surface targeting motif at the C-terminus of Kv1.3, KCNE4 subunits retain Kv1.3 in the ER (145, 146).

Associated receptors and scaffold proteins

Integrins are a family of cell surface receptors that mediate cell-cell and cell-matrix interactions. They are heterodimers formed by noncovalent association of α and β subunits, each formed by a large extracellular domain (> 1500 aa), a single transmembrane helix and a short cytoplasmic tail. Each integrin has a large repertoire of specific extracellular ligands and cell surface receptors, including ion channels (3, 11). Integrin receptors coordinate bidirectional signaling, regulating cellular functions such as cell growth and differentiation, cell activation, adhesion and migration. By linking ECM molecules to actin cytoskeleton, they control cell responses as a function of the extracellular environment (the "outside-to-in" signaling). Also, their binding to extracellular ligands is modulated by interactions with intracellular signals and proteins ("inside-to-out" signaling) (3, 61, 62).

Physical and functional association of integrin with Kv1.3 channels was first reported in T-cells. β1 integrin mediates T-cell adhesion and migration following activation of Kv1.3 with different stimuli (97). Kv1.3 opening by membrane depolarization activates integrin signaling, an example of the "inside to out" mechanism. A physical link between Kv1.3 channels and β1 integrins has also been found in melanoma cells (4). The "outside to in" signaling (integrin activation modulates ion channel) has been recently described for hERG channels (9). In fact, when associated with integrins, ion channels function becomes bidirectional as well. These reciprocal regulatory interactions can rely on subtle structural rearrangements (as voltage-dependent conformational changes), driving a change that mechanically propagates across the bilayer. K^+ channels response to changes in transmembrane potential includes changes in the location and the coupling of cytoplasmic loops and helices. As there is a tight coupling between the lipid environment and the conformation of the protein, these changes can affect the conformation of other proteins in the same lipid raft. In these cases neither a stable physical association nor ion fluxes will be required for the coupling between the channel protein and the integrin receptor (3, 63).

Cortactin is a F-actin binding protein that upon phosphorylation-mediated activation promotes polymerization and rearrangement of the actin cytoskeleton. Cortactin is a cytoplasmic protein with an amino-terminal acidic region followed by 6.5 tandem repeats responsible for F-actin binding (58) and a carboxy-terminal proline-rich region with several phosphorylation sites and a SH3 domain (85). The N-terminus is critical for regulating branched actin assembly while the C-terminus allows cortactin to function as a scaffold. Many proteins bind to the SH3 domain and in this way can be bridged to the actin cytoskeleton (48, 55). Cortactin represents a good candidate to mediate cytoskeleton modulation of ion channels function, as it can bind and regulate ion channels, and in particular Kv channels (33, 68, 72, 169). In T-cells cortactin and Kv1.3 interaction takes place upon activation of the immune synapse (the specialized junction between the T-cell and the antigen presenting cell, APC). However, binding of Kv1.3 C-terminus with the SH3 domain has also been shown in HEK cells (68). These results suggest that actin dynamics regulates the membrane mobility of Kv1.3 channels, contributing to Kv1.3 localization.

PDZ proteins. Proteins containing PDZ domains anchor membrane receptor proteins to cytoskeletal components, holding together and organizing signaling complexes at the membrane. Interaction of Kv1 channels with adaptor PDZ-containing proteins of the MAGUK family was first reported in neurons (84). The PDZ domains enable protein-protein interaction with multiple partners, regulating the activity, the transport and clustering of the channels at the membrane and supporting the assembly of macromolecular complexes.

Kv1.3 channels associate with two different PDZ proteins: PSD95 (or Dlg4) and SAP97 (or Dlg1), through a PDZ-binding motif located at the C-terminus (107, 155). The interaction of SAP97 with Kv1.3 was first described in T-cells (69). SAP97 binds both Kv1.3, and the tyrosine kinase Lck, allowing the physical proximity between them that is required for Kv1.3 current downregulation by protein kinase A (91). PSD95 association with Kv1.3 can affect both channel location (promoting clustering of the channels at specific membrane domains) and channel activity through the PSD95 SH3-gk domain. In Jurkat cells both PSD95 and SAP97 interact directly with Kv1.3, but only PSD95 participates in Kv1.3 targeting (155). Altogether, it seems that while association with SAP97 regulates Kv1.3 channel phosphorylation, association with PSD95 determines mainly Kv1.3 location (91)

Caveolins. Caveolae are a type of lipid raft, defined by the presence of the cholesterol binding proteins caveolins. Caveolins are integral membrane proteins with cytoplasmic amino- and carboxy-terminal domains, which emerge as signaling platforms creating a cluster of lipids and proteins where effector proteins and their substrates interact. (70). Kv1.3 channels present a caveolin-binding domain (FQRQVWLLF) at the cytoplasmic N-terminal domain. Targeting of Kv1.3 to caveolar rafts reduces the channel´s lateral mobility, increases Kv1.3 currents and enhances C-type inactivation (131) whilst disrupting the rafts inhibits channel activity (20). Interestingly, the presence Kv1.5 could impair Kv1.3 targeting to caveolae. In macrophages Kv1.3/Kv1.5 ratio changes in response to stimulation. This change regulates the targeting of the channels to microdomains enriched in signaling molecules and consequently their functional response (164, 166).

Post-translational modifications

Covalent post-translational modifications such as glycosylation, palmitoylation or phosphorylation regulate ion channel functions, either directly or indirectly by modulating their interaction with adaptor proteins. Protein phosphorylation is the most common post-translational modification.

Regulation of Kv1.3 currents by phosphorylation was first described in the early 90`s in T-cells by serine-threonine kinases (reviewed in (122). Kv1.3 channels are also regulated by phosphorylation on tyrosine residues, through both receptor and non-receptor tyrosine kinases (TK). Tyrosine phosphorylation of Kv1.3 channels, after activation of either receptor TK (such as EGFR) or non-receptor TK such as Src, decreases Kv1.3 currents (75). Several aspects emphasize the complexity of this modulation: first, multiple tyrosine residues can be involved in the modulation of Kv1.3 (56). Second, Kv1.3 channel activity can be differentially modulated by

several TK receptors (45). Third, the effects of TK can be modulated by adaptor proteins within the Kv1.3 channelosome (47). These associations can change the channel responses and can also lead to a reciprocal modulation of TK expression and activity by the channel itself (46).

Kv1.3 channels are part of multiprotein signaling complexes whose functions go beyond anchoring the channel to the membrane. These complexes present some clear advantages, including the increased efficiency of the enzymatic reactions (as both the substrate and the effector have a restricted diffusion), the compartmentalization that may be essential for determining specificity in the signaling pathways and the increasing speed that can be necessary for the transmission of the signals that regulate some channels.

Kv1.3 channels and proliferation

<u>The immune system</u>

T lymphocytes. Long before the molecular nature of ion channels was unraveled, Kv1.3 currents were described and functionally characterized in T-cells (25). Also in this preparation a role of Kv1.3 channels in mitogenesis was proposed for the first time (49). Thus, it is not surprising that most of our knowledge regarding the kinetic and pharmacological properties of Kv1.3 channels, their molecular associations, their contribution to cellular homeostasis and their role in the pathways leading to activation and proliferation has been characterized in T lymphocytes.

The generation of an efficient immune response against an antigen requires clonal expansion of lymphocytes. Antigens are presented to T-cells by professional antigen presenting cells (APCs) such as B-cells, dendritic cells or macrophages. Interaction of the antigens with the T-cell receptor/CD3 complex (TCR/CD3) activates signaling cascades involving TK pathways. Some of these pathways such as the Ras/MAPK pathway can initiate the expression of genes required for T-cell proliferation through PLC activation and the production of IP3 and DAG. DAG activates PKC which phosphorylates and activates several transcription factors such as NFκB. Meanwhile, IP3 production evokes a biphasic increase in $[Ca^{2+}]_i$ by releasing it from intracellular stores and by activating a sustained Ca^{2+} entry through “Calcium Release-Activated Ca^{2+} channels” (CRAC). CRAC channels activate in response to Ca^{2+} store depletion. In T-cells ORAI1 and STIM1 are the genes encoding this calcium entry pathway (26, 59, 129). The Ca^{2+} signal also contributes to the activation of the transcription factor NFAT (nuclear factor of activated T-cells), which binds to the promoter of the interleukin-2 (IL-2) gene. Upon IL-2 gene activation T-cells are committed to proliferate even in the absence of the antigen.

Kv1.3 channels and the intermediate conductance Ca^{2+}-activated K^+ channels (KCa3.1 channels) are the main K^+ conductances in T-cells. Their activation, either by membrane depolarization (Kv1.3) or by increases in intracellular Ca^{2+} (KCa3.1) leads to membrane hyperpolarization, increasing the driving force for Ca^{2+}. These two channels determine T-cells

resting V_M, thus regulating the magnitude of the Ca^{2+} signal required for gene transcription and cell proliferation.

Upon stimulation by APCs, naïve T (T_N) cells upregulate the expression of KCa3.1. Following antigen clearance, >95% of the effector cells die while a small pool ultimately develops into long-lived memory T-cells. A single T_N cell can generate many subsets of memory T-cells with different functional properties and gene expression profiles. At least two clonally expanded populations emanate from this primary response, ones expressing C-C chemokine receptor 7 ($CCR7^+$), that lack immediate killing activity, and $CCR7^-$ cells that are capable of immediate cytotoxicty. $CCR7^+$ cells are named central memory T-cells (T_{CM}) because of their location in secondary lymphoid tissues, while $CCR7^-$ cells were named effector memory (T_{EM}) because of their rapid effector function ex-vivo (106). An important difference between them is the K^+ channel expression pattern. Upon activation, T_N and T_{CM} cells upregulate the expression of KCa3.1, while T_{EM} upregulate the expression of Kv1.3 channels. Hence T_{EM} cells are highly dependent on Kv1.3 channels for Ca^{2+} signaling and cytokines production. In the presence of selective Kv1.3 blockers, they fail to migrate, proliferate and secrete cytokines. As T_{EM} cells participate in the pathogenesis of autoimmune diseases (6, 14, 31), these differences in K^+ channel expression represent a great therapeutic opportunity for the use of Kv1.3 blockers.

B lymphocytes. Although less extensively studied, B lymphocytes show also dramatic changes in K^+ channel phenotype during differentiation and activation, paralleling T-cell changes. Quiescent T_N and early memory B-cells upregulate KCa3.1 upon activation, while late memory cell of the T (T_{EM}) and B (class-switched) lineages increased Kv1.3 channels when activated (172).

Natural Killer (NK) cells. NK cells participate in both innate and adaptive immune responses and play a major role in the defense against tumors and virus. Human NK cells express both Kv1.3 and KCa3.1 channels, but their expression varies between the different subsets of NK cells. As for B and T lymphocytes, these differences are important in regulating V_M, degranulation and proliferation of the NK cells subpopulations, that can be differentially targeted with selective blockers (87).

Macrophages, Dendritic Cells (DCs) and Monocytes. The responses of these cells are initiated by recognition of pathogen-associated molecular patterns (PAMPs) through receptors such as toll-like receptors (TLRs). Activation of these receptors induces proinflammatory cytokines that can in turn activate macrophages and DCs. Kv1.3 and Kv1.5 have been described in macrophages, most likely as heterotetramers (165, 166). Kv1.3 channel expression has been reported to increase upon activation and differentiation in response to inflammatory injury in macrophages, and its blockade inhibits migration and proliferation (163).

The nervous system

Kv1.3 channels are expressed in the mature nervous system in several subsets of neurons and in all types of glial cells: astrocytes, oligodendrocytes, and microglia. In contrast to neurons, glial

cells undergo proliferation in response to physiological or pathological cues, and a role for Kv1.3 channels has been proposed in this response.

Astrocytes. While initially they were considered only as *gap fillers* among neurons, astrocytes are now thought to play a number of active roles in the brain, including transmitter uptake and release, blood flow regulation, maintenance of an appropriate neuronal environment, modulation of synaptic transmission and nervous system repair (2). They respond to insults through a process named reactive astrogliosis, which is a pathological hallmark of CNS structural lesions. However, the contribution of astrocyte proliferation to reactive astrogliosis is controversial. In healthy CNS tissue, astrocyte turnover is very low. Few actively dividing reactive astrocytes can be found after brain injury, and their source is not well established (7).

Changes in K^+ channels expression have been implicated in the injury-induced proliferation of astrocytes, but their molecular correlates are not fully characterized. Within a few hours after the injury, downregulation of inward rectifier K^+ currents (K_{IR}) with concurrent upregulation of outwardly delayed rectifier K^+ (K_{DR}) currents has been described. K_{DR} currents increase in proliferating astrocytes and decrease with cell cycle exit and differentiation. Blockade of K_{DR} currents with TEA or 4-AP inhibited glial proliferation and retarded scar repair, supporting their contribution to proliferation (104, 105). Kv1.5 has been proposed to mediate this proliferation-dependent K_{DR} current, by a mechanism involving Src-mediated Kv1.5 phosphorylation (104).

Oligodendrocytes. The adult nervous system has a very limited capacity of neural replacement. However, oligodendrocytes regenerate neuronal myelin membranes in response to a wide variety of insults in a rapid and efficient way. This remarkable repair capacity relies on the presence of oligodendrocyte precursor cells (OPCs) in the adult CNS, with phenotypic characteristics of immature oligodendrocytes (168). While activation of OPCs can be observed in response to inflammatory or immunological insults, the proliferative response is only observed in response to demyelination or generalized tissue destruction (15).

The expression of K_{DR} channels correlates with the proliferative potential of oligodendrocytes. OPCs exhibit large Kv currents, that are absent in postmitotic oligodendrocytes (5, 37). In proliferating OPCs there is an increase in the synthesis of both Kv1.3 and Kv1.5 mRNA during G_1 phase of the cell cycle that is accompanied by an up-regulation of outward Kv currents. Currents through Kv1.3-containing channels seem to play a crucial role in G_1/S transition, as Kv1.3 blockade inhibits S-phase entry (37). In contrast, the functional contribution of Kv1.5 channels to proliferation is unclear, since Kv1.5 antisense oligonucleotides reduced OPCs Kv1.5 protein expression and Kv current density with no effect on proliferation (5).

Several results indicate a good correlation between the functional expression of Kv1.3 currents and OPCs proliferation: 1) selective Kv1.3 blockers inhibit OPCs proliferation; 2) in acute isolated cells, Kv1.3 expression decreases as OPCs progress through the lineage becoming less proliferative (37); 3) overexpression of Kv1.3 channels in OPCs mimics the effect of mitogens like PDGF, suggesting that Kv1.3-channels are essential mediators of PDGF-driven

proliferation (159). In this regard, it has been shown that PDGF-induced increase of K_{DR} in OPCs cells is sensitive to TK inhibition (36).

Other Kv channels apart from Kv1.3 have been described to regulate OPCs proliferation, either as activators (as Kv1.4) or inhibitors, as for Kv1.6 (159). The mechanisms for these synergic or antagonic effects on proliferation are poorly understood.

Microglial cells. They are the primarily scavenger cells of the CNS, sharing many properties with macrophages. They represent the first line of defense in CNS, with functions that include phagocytosis of cellular debris, regulation of astroglyosis, neutralization of infectious agents and coordination of neuroimmune responses. Microglia activation involves a stereotypic pattern of responses: increased expression of immunomolecules, proliferation, recruitment to the site of injury and release of inflammatory mediators (60). K^+ channels participate in several of these responses, but it is hard to unravel their specific role(s) in each aspect of microglial function, including proliferation, secretion, migration or chemokine release. Nevertheless, K^+ channel upregulation is a hallmark of microglial activation (137). It has been suggested that unstimulated microglia cells express only K_{IR} channels, but upon activation there is an increased expression of Kv channels (117), mainly Kv1.5 and Kv1.3 channels. The seminal study of Kotecha and Schlichter (88) showed expression of both Kv1.3 and Kv1.5 proteins in cultured microglia cells. Both currents were involved in microglia proliferation but their relative roles changed with time in culture. Quiescent microglia cells expressed predominately Kv1.5 currents whilst Kv1.3 currents become predominant upon proliferation.

Similar results are reported in other models of microglial cells proliferation. Deletion of Kv1.5 channels increased microglia proliferation upon facial nerve axotomy, and cultured microglia treated with Kv1.5 antisense oligonucleotides showed an increase proliferation rate (121).

However, proliferation represents only an intermediate level of activation. The induction of the expression of both Kv1.3 and Kv1.5 channels is required for full microglial activation, albeit each channel plays a different role. Upregulation of Kv1.5 channels is required for increased nitric oxide production, but leads to cell cycle arrest, while Kv1.3 channels do appear to have a role in microglial proliferation and migration as well as in cytokine release (32, 149). Finally, simultaneous upregulation of both channels have been suggested in the case of stimulation of microglia with lipopolysaccharide (LPS), which increases a Kv current with biophysical properties compatible with heterotetrameric Kv1.5/Kv1.3 channels (121). LPS stimulation induces cytokine release, but decreases proliferation.

These observations have also therapeutic implications. If Kv1.5 is more important for resting cells and Kv1.3 is required for proliferating microglia, it may be possible to downregulate overactive microglia without compromising the functions of resting microglia.

Vascular smooth muscle cells

Vascular smooth muscle cells (VSMCs) in the vessel wall retain a considerable degree of plasticity, being able to switch from a quiescent contractile state to a synthetic, migratory and

proliferative one, in response to chemical, mechanical or inflammatory stimuli (119, 167). This functional change of VSMCs is known as phenotypic modulation (PM). VSMC plasticity contributes to the development and homeostasis of blood vessels, but it has also ill-fated consequences as it is central to the process of intimal hyperplasia, a pathological lesion in vascular diseases displaying unwanted remodeling (112). PM of VSMCs during vascular diseases involves activation, proliferation, migration and dedifferentiation of VSMCs, and in all these events the contribution of different ion channels and transporters is also required.

A large number of ion channels, including several K^+ channels have been involved in the PM of VSMCs (12, 35, 79, 89, 110, 114). In a large-scale analysis of the changes of ion channels expression upon PM both *"in vitro"* and *"in vivo"* models, only two ion channel genes were up-regulated in both models: Kv1.3 channel and its accessory subunit Kvβ2.1 (42). Proliferating VSMCs exhibited increased Kv1.3, and Kv1.3 selective blockers inhibited cultured VSMCs migration and proliferation (35, 42). This effect has been also demonstrated in "*in vivo*" proliferative models (35, 43). As the increased contribution of Kv1.3 channels associates to a decreased expression of Kv1.5 channels, the Kv1.3/Kv1.5 ratio has been proposed as a marker of VSMC phenotype (40, 41). Interestingly, Kv1.3 and Kv1.5 channels have opposite effects on proliferation when overexpressed in heterologous expression systems: Kv1.3 channels increased HEK cells proliferation while Kv1.5 channels decreased it (40).

Kv1.3 channels and proliferation: Mechanisms

Ion channels can regulate many signaling pathways involved in proliferation by modulating the electrical, mechanical and chemical properties of the cells. The complexity of this regulation explains why data on the signaling pathways activated by ion channels often remain described at a phenomenological level. We will try to dissect some of the potential mechanisms participating in the crosstalk between Kv1.3 channels and the biochemical pathways linked to proliferation, using data from the three "model" systems described previously (T-cells, glial cells and VSMCs). Initially, we can consider two non-exclusive mechanisms: either cells use Kv1.3 channels to regulate V_M and hence cell cycle, or Kv1.3 channels serve as sensors of changes in V_M, acting as signaling molecules connecting bioelectrical membrane signals with intracellular biochemical pathways. In other words, we can speculate that Kv1.3 is important in proliferation because it is a K^+ channel that regulates V_M or because is a membrane protein that can undergo conformational changes in response to changes in V_M. In fact, it is very likely that both mechanisms contribute to proliferation, as their individual participation is difficult to dissect. Moreover, evidence points to the importance of Kv1.3 channelosome in the proliferative response, and again, signaling through these closely associated proteins can be dependent on K^+ fluxes (permeation) or conformational changes (gating).

Kv1.3 as K^+ channels: the membrane potential model

In non-excitable tissues, Kv channels regulate V_M and cell volume, and both processes have been linked to cell cycle progression. Two complementary observations have led to the

proposal that Kv channel activity is crucial for cell cycle progression: 1) Kv channel blockers inhibit proliferation whilst channel openers stimulate it, and 2) Kv channel expression and/or activity is upregulated by mitogens (170). Accepting this causal relationship between Kv channel activity and cell proliferation, two different hypotheses have been put forward to expound the underlying mechanism.

The first one proposes that Kv channels modulate cell cycle progression by controlling resting V_M. K^+ conductance govern resting V_M in all cells, so that changes in K^+ channel activity would determine cell cycle oscillations of V_M. Current models of these changes outline a rhythmic, slow oscillation of V_M with a hyperpolarizing spike occurring before S phase initiation and a prolonged depolarization being required for the G2/M transition (19). The resulting changes in V_M can regulate for instance Ca^{2+} fluxes. Also, regulation or recruitment of signaling molecules to the membrane can be dependent on V_M (96).

In the second hypothesis, the important signal contributing to proliferation will be K^+ efflux upon Kv channel activation. Although the mechanism is not well described, cell proliferation is influenced by cell volume, which is in turn dependent of ion and water fluxes (reviewed in 72, 90). A bell-shaped relationship between cell proliferation and volume has been defined experimentally, indicating that proliferation is optimal within a cell volume window. This model could describe any change in rate of proliferation, suggesting a phenomenological correlation between cell volume and division. In this context, the cell volume increases upon K^+ channels inhibition will be the mechanism responsible for decreased proliferation (53).

The contribution of Kv1.3 (and KCa3.1) channels to T-cell mediated activation and proliferation is related to its prominent role controlling V_M and indirectly, Ca^{2+} driving force (Figure 2). The contribution of these channels to resting V_M will vary depending on the T-cell subtype and on its activation state (see above). The voltage-dependence of activation and inactivation of Kv1.3 determines a membrane potential window at which the ion channels are active, while KCa3.1 channels are mostly silent at rest. This, together with the high input resistance of the membrane of unstimulated T-cells allows effective V_M control by a reduced number of channels with the resting conductance dominated by Kv1.3 channels (156, reviewed in (23, 122).

Control of V_M does not require a particular spatial distribution of channels in the membrane of T cells. However, Kv1.3, KCa3.1 and CRAC channels are recruited to the immunological synapse during antigen presentation (115, 116, 124, 136) which most likely provides additional signaling functions. Concentration of CRAC channels will produce a high localized $[Ca^{2+}]_i$ within nanodomains close to the APC, which can trigger localized Ca^{2+} binding or activation of enzymes or other signaling proteins. As mitochondria also accumulate in the subsynaptic region, they remove Ca^{2+} and contribute to maintain CRAC current. The accumulation of Kv1.3 and KCa3.1 channels at the synapse facilitates the clustering and modulation of associated proteins. It will also result in a localized increase in $[K^+]_e$ upon channels activation. The subsequent membrane depolarization will favor Kv1.3 activation, but there are also direct effects of increase $[K^+]_e$ on Kv1.3 channels, including augmented single channel current and

decreased inactivation (24), that together with membrane depolarization will tend to increase Kv1.3 channel activity. This augmented activity of Kv1.3 dependent on $[K^+]_e$ is tightly regulated. Larger increases in $[K^+]_e$, (as in necrotic areas within solid tumors), lead to an inhibition of T-cell activation, that is independent on the effect of $[K^+]_e$ on V_M but depends upon changes in $[K^+]_i$ (54).

Kv1.3 as voltage-dependent proteins: The voltage sensor model.

In addition to allowing ion permeation, ion channels can also have non-conducting functions that enable them to interact with cell signaling pathways and directly regulate biochemical events (80). Certain types of ion channels such as the transient receptor potential channels (TRPs) are truly bifunctional proteins, with protein kinase domains in their cytosolic domains (139). Although no enzymatic activity has been described for the Kvα subunits, such activity could be present on the associated molecules within the channelosome. In fact, the macromolecular complex includes accessory subunits such as Kvβ, that show enzymatic activity (66). Therefore, the proteins within these complexes may contribute to this double life of Kv channels by transducing voltage-dependent conformational changes into cytoplasmic or nuclear signals affecting proliferation. The studies dissecting the contribution to the cell cycle of either conducting or non-conducting properties of Kv channels are scarce. As mentioned, it is now widely recognized that even though ion fluxes through the channel can also participate in this response, the final outcome is the triggering of a signaling pathway that other types of channels with similar electrical properties cannot recapitulate (80, 95).

The effect of Kv1.3 on proliferation in native cells was reproduced upon heterologous expression in HEK293 (40). Moreover, while Kv1.3 increases HEK proliferation, Kv1.5 has the opposite effect. These antagonistic effects give support to the idea that concurrent changes in the expression of these two channels in the native systems (discussed below) are in fact a requirement for the functional changes associated with dedifferentiation and proliferation. These observations also widened the possibilities for exploring the mechanism through which Kv1.3 increases proliferation. Several extreme Kv1.3 mutant channels have been used to determine the role of permeation, voltage sensor and channel location on proliferation. Non-conducting mutants induced proliferation to the same extent than WT channels. However, the effect was lost in voltage-insensitive channels or channels not expressed at the plasma membrane (40), indicating that Kv1.3 channels only need their voltage sensing capability to impact proliferation, while V_M modulation by Kv1.3 channels is nonessential. Electrical signals (V_M changes during cell cycle) are converted into a biochemically supported transduction pathway in the Kv1.3 molecule, whose conformational changes activate proliferation signaling (Figure 3). In fact, absolute values of V_M in HEK cells are irrelevant for proliferation since wild type and non-conducting Kv1.3 channels show a similar effect on proliferation with a very different effect on V_M, while Kv1.3 and Kv1.5 have similar effects on V_M and opposite effects on proliferation (40).

Effects on proliferation of non-conducting channel mutants have been also described for another K^+ channels, such as Eag1 and Ca^{2+}-activated K^+ channels (71, 111). However, in all

cases these data were obtained in heterologous systems, due to the clear difficulties to break up gating and permeation of native channels. Nevertheless, there are some clues suggesting that the pro-proliferative effect of Kv1.3 in VSMCs may not be simply related to its role as a K^+ channel. In femoral VSMCs the contribution of Kv1.3 channels to resting V_M does not recapitulate the effects of Kv1.3 on VSMC proliferation. Kv1.3 blockade leads V_M depolarization and inhibits proliferation. However, when depolarization was induced by blockers of other K^+ channels or by high $[K^+]_e$, cell proliferation was unaffected (42). Moreover, proliferation inhibition by Kv1.3 blockade was still present in VSMCs incubated in high (20 mM) $[K^+]_e$, which suggest a role for Kv1.3 channels in VSMCs proliferation beyond membrane depolarization.

These findings open interesting questions for future research. First, they indicate that the original hypothesis of depolarization inducing growth (18) is too simple and then, probably inaccurate. Although the general idea that cell cycle progression is marked by changes in V_M still holds true (110) the relationships between cell cycle stages and V_M are complex, because 1) V_M variations are cell-type specific, 2) the control of V_M is highly non-linear as many of the proteins that determine V_M are themselves voltage-regulated and 3) the expression and function of ion channels may fluctuate along the cell cycle (10, 110, 158). Second, if Kv1.3 behaves only as a sensor, and the changes in V_M needed to proceed along cell cycle are not due to its activation, we need to identify the channel(s) and the signals responsible for cell cycle oscillations of V_M. An attractive hypothesis linking changes in V_M to G1 restriction point associates changes in V_M with the polyamine synthesis occurring before entry in the S phase, since several K^+ channels are known to be inhibited by these molecules (128). The change in V_M would be an indirect check of the correct increase in polyamine needed for DNA packing, and Kv1.3 sensing of V_M changes could facilitate cycle progression. Third, if K^+ flux is not a requirement, then the voltage-dependent conformational change of Kv1.3 needs to be transduced via associated proteins within the Kv1.3 channelosome. The molecular identification of the putative docking sites in the Kv1.3 molecule, the nature of the interacting molecules, and the mechanisms involved need to be identified.

The opposite effect of Kv1.3 and Kv1.5 on HEK proliferation provided a useful tool to scrutinize the molecular determinants of Kv1.3-induced proliferation. Kv1.3-Kv1.5 chimeras indicate that these molecular determinants located exclusively on Kv1.3 C-terminus (78). Alanine mutation of consensus phosphorylation residues showed that Kv1.3-induced proliferation was fully abolished by three point mutations, two of them located in a short region near the S6 helix (the YS-segment). Remarkably, YS segment recapitulates Kv1.3-induced proliferation in a Kv1.5 backbone, but only at one specific location. Altogether, it seems that voltage-induced conformational changes of Kv1.3 channels regulated the accessibility of these phosphorylation sites, which function as specific docking sites to activate proliferation signaling cascades. Channel phosphorylation increases with the closed to open transitions and decreased in voltage-insensitive channels, which reside in the inactivated state (78).

Kv1.3 as multiprotein complexes: the channelosome model

In Kv1.3 KO mice, the levels of various signalling molecules (TK, adaptors, PDZ-containing proteins…) are significantly elevated in olfactory bulbs (57). In addition, the synaptic glomeruli of these animals show profound effects on development that are not seen in the absence of other ion channels that are also expressed in olfactory neurons such as Kv3.1 (73). Kv1.3 channel seems to be a central signalling molecule as it appears unlikely that these effects of Kv1.3 absence can be attributed simply to changes in membrane excitability. Nevertheless, it is conceivable that the complete absence of a channel protein will lead to a drastic reorganization of the associated proteins, including modulatory subunits, scaffolds, receptors or cytoskeletal elements. They all can participate in Kv1.3 signaling, and for many of them such roles have been described. Additionally, changes in the distribution, the location or the association between Kv1.3 and some of these interacting proteins may contribute to the proliferative responses. To explore these aspects it is useful to study systems where cells switch from quiescent to proliferative phenotypes. Activation of proliferation in normally post-mitotic cells can be particularly useful to explore the underlying mechanisms, because as shown for microglia activation, they do not necessarily recapitulate the normal *"in vivo"* switch during early postnatal development (88).

With the notable exception of T-cells, in all the systems described so far cells express other Kv1 channels, whose expression is also remodeled upon activation and/or proliferation (42, 83, 149, 159). Notably, associated changes in Kv1.5 channels (often decreased expression) accompany Kv1.3 upregulation in proliferative cells. This is the case in OPCs (5), in microglial cells (88) and VSMCs (40, 41). The relative amount of homo or heterotetrametric Kv1 channels could be relevant. In fact, a very attractive hypothesis is that the master switch determining Kv1 effect on proliferation is not the upregulation of Kv1.3 channels but the control of the Kv1.3 to Kv1.5 ratio. In most VSMCs preparations, PM does not change the expression of Kv1.3 mRNA (41, 43). However, all other Kv1.x mRNA decreased upon proliferation. This decrease is particularly drastic for Kv1.5, which changes from being the most abundant Kv1 transcript in quiescent cells to almost disappear in proliferating cells (40, 42).

The implications for cell proliferation will be different depending on whether the response depends upon Kv1.3 conformational changes or whether Kv1-mediated fluxes are also necessary. Biophysical implications of a switch from Kv1.5 to Kv1.3 current include their different voltage dependencies. The resulting window currents will determine the ability of the cells to oppose both brief and long depolarization influences resulting from activation of other receptor or channels (39, 82, 113, 140). Lack of C-type and cumulative inactivation in Kv1.5 will result in an increased channel availability after repetitive stimulations. Also, if Kv1.3 and Kv1.5 have also roles in proliferation beyond their K^+ permeability, both channels might be subject to different regulations depending on their location and their associated proteins.

A more complex scenario arises if we consider that Kv1.3 and Kv1.5 are likely to form heteromultimeric channels, like other Kv1 channels (133, 144, 151, 160), so that a range of possible Kv currents may be observed. However, regarding modulation by associated proteins,

these complexes could behave as dominant negative, so that the interaction(s) of either subunit with other scaffolds, enzymes or receptors will be hindered in the multimeric channel (Figure 4). In microglial cells, based on confocal images, Kv1.3/Kv1.5 heteromultimers seemed unlikely in quiescent cells (showing very little Kv1.3 immunoreactivity and largely intracellular), although their proportion could increase as the cells started to proliferate (88). At latter times in culture, the cells express Kv1.3 like currents, while Kv1.5 immunoreactivity decreased and remains mainly intracellular. The presence of heterotetramers Kv1.3/Kv1.5 has also been observed in microglial cells stimulated with LPS (121).

Heterotetrameric Kv1 channels are present in quiescent VSMCs (34, 133), but not in proliferating VSMCs, where only Kv1.3 currents are detected. Kv1.3 behaves as a housekeeping gene in VSMCs, as its expression cannot be stimulated by mitogens (41). It is conceivable that Kv1.5 downregulation could be the relevant change for VSMC proliferation, thus if Kv1.5 decrease is prevented PM will not take place. In this case, the role of Kv1.5 could be the activation of anti-proliferative signaling or the occlusion of the pro-proliferative Kv1.3 signals by Kv1.3/Kv1.5 heterotetramerization. In support of the first option, heterologous expression of Kv1.5 alone reduces proliferation (40). However, it is not clear whether this will be the case in native systems. In microglial cells, an antiproliferative role of Kv1.5 has been described since Kv1.5 antisense oligonucleotides increases proliferation. Using an *"in vivo"* model of microglia activation, LPS treatment decreased proliferation rate in WT animals but not in Kv1.5$^{-/-}$ animals (121). Notwithstanding, as Kv1.3 channels are also expressed in these cells, the antiproliferative role of Kv1.5 could also be explained by the occlusion of Kv1.3-induced proliferation. Kv1.5 overexpression in cultured VSMCs shows a decrease of proliferation rate similar to that observed with Kv1.3 blockers, and the two effects are non-additive (unpublished data). The interference of Kv1.5 with the pro-proliferative signaling pathways activated by Kv1.3 is the simplest explanation for these observations.

Concluding remarks/Future directions

The work of many laboratories over the past decades points to a relevant role of Kv1.3 channels in the proliferative response of different cell types under physiological conditions. This finding is relevant as it provides novel therapeutical targets to prevent unwanted proliferation. It also adds tools to investigate the molecular processes regulating cell proliferation that are dependent on Kv1.3 channel function. In this regard, while the contribution of Kv1.3 to cell proliferation seems to be a shared feature of many cell types, the underlying processes linking Kv1.3 expression and/or activity to cell cycle progression are diverse and far from being fully understood (Figure 5). The diversity of mechanisms reflects the complexity of the functions carried out by Kv1.3 channels. Ion channels are pore-forming macromolecular complexes that allow the passive ion flow across membranes. However, they are not simple ionophores, but also integral membrane proteins that can mediate key steps in signal transduction in an exquisitely channel- and tissue-specific manner. In the case of voltage-gated channels, they can also act as transducers of bioelectrical signals into biochemical cascades. Because Kv1.3 channels are regulated by V_M

and in turn they regulate V_M, bioelectrical signals can be highly non-linear, leading to complex recursion of the effects.

Still, the interactions of Kv1.3 channels with V_M are just one of the best known and more easily controlled cellular bioelectrical parameters. Subcellular organelles also have their own transmembrane potential, due to specific expression of ion channels. Kv1.3 channel expression is well documented in mitochondria, where they modulate cancer cells growth and apoptosis by regulating mitochondrial transmembrane potential (94, 153, 157). Mitochondrial Kv1.5 channels decrease associates with increased proliferation, while restoration of Kv1.5 channel led to apoptosis (22). It is tempting to speculate that the Kv1.3/Kv1.5 ratio at intracellular organelles could be also an important modulator of proliferation. The contribution of these subcellular altered bioelectrical signaling to cell cycle control warrants further research.

Many different K^+ channels have been shown to regulate cell proliferation, highlighting the fact that K^+ channel function, by modulating processes like membrane potential, cell volume and Ca^{2+} influx, is an important element in cell cycle regulation. This could be the case for Kv1.3 channels in T-cell activation and proliferation. Still, we tend to define K^+ currents as the only effectors, ignoring other possible actions of the channel molecule itself. The specific requirement of Kv1.3 for proliferation in several cell types argues against this belief, suggesting that other permeation-independent mechanisms are also involved. Non-canonical functions can influence cell proliferation in the absence of (or in addition to) K^+ efflux. This scenario could help to explain the proliferation-associated changes in Kv channels expression observed in several preparations (oligodendrocytes, microglia, VSMCs). In these cases the need of a dominant expression of Kv1.3 channels supports the involvement of molecule-specific interactions via mechanisms that are just starting to be identified. While heterologous expression studies suggest that Kv1.3-induced proliferation does not require K^+ fluxes, more research is needed to extrapolate these observations to native tissues, where the contributions of canonical and non-canonical channel functions are more difficult to dissect. In addition, the differential effects on proliferation of Kv1.3-containing heteromultimeric channels described in some preparations adds complexity but also opens fascinating possibilities for the fine-tune control of cell proliferation.

References

1. **Accili EA**, **Kuryshev YA**, **Wible BA**, **Brown AM**. Separable effects of human Kvbeta1.2 N- and C-termini on inactivation and expression of human Kv1.4. *J Physiol* 512: 325-36, 1998.
2. **Araque A**, **Parpura V**, **Sanzgiri RP**, **Haydon PG**. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci* 22: 208–15, 1999.
3. **Arcangeli A**, **Becchetti A**. Complex functional interaction between integrin receptors and ion channels. *Trends Cell Biol* 16: 631–639, 2006.
4. **Artym V V.**, **Petty HR**. Molecular Proximity of Kv1.3 Voltage-gated Potassium Channels and β1-Integrins on the Plasma Membrane of Melanoma Cells. *J Gen Physiol* 120, 2002.
5. **Attali B**, **Wang N**, **Kolot A**, **Sobko A**, **Cherepanov V**, **Soliven B**. Characterization of delayed rectifier Kv channels in oligodendrocytes and progenitor cells. *J Neurosci* 17: 8234–8245, 1997.
6. **Azam P**, **Sankaranarayanan A**, **Homerick D**, **Griffey S**, **Wulff H**. Targeting effector memory T cells with the small molecule Kv1.3 blocker PAP-1 suppresses allergic contact dermatitis. *J Invest Dermatol* 127: 1419–29, 2007.
7. **Barreto GE**, **Gonzalez J**, **Torres Y**, **Morales L**. Astrocytic-neuronal crosstalk: Implications for neuroprotection from brain injury. *Neurosci. Res.* 71: 107–113, 2011.
8. **Barros F**, **Domínguez P**, **de la Peña P**. Cytoplasmic domains and voltage-dependent potassium channel gating. *Front Pharmacol* 3 MAR: 1–15, 2012.
9. **Becchetti A**, **Crescioli S**, **Zanieri F**, **Petroni G**, **Mercatelli R**, **Coppola S**, **Gasparoli L**, **D'Amico M**, **Pillozzi S**, **Crociani O**, **Stefanini M**, **Fiore A**, **Carraresi L**, **Morello V**, **Manoli S**, **Brizzi MF**, **Ricci D**, **Rinaldi M**, **Masi A**, **Schmidt T**, **Quercioli F**, **Defilippi P**, **Arcangeli A**. The conformational state of hERG1 channels determines integrin association, downstream signaling, and cancer progression. *Sci Signal* 10: eaaf3236, 2017.
10. **Becchetti A**, **Lehen'kyi V**, **Shapovalov G**, **Skryma R**, **Prevarskaya N**. Ion channels and transporters in cancer. 1. Ion channels and cell proliferation in cancer. *Am J Physiol Cell Physiol* 301: C255–C265, 2011.
11. **Becchetti A**, **Pillozzi S**, **Morini R**, **Nesti E**, **Arcangeli A**. New insights into the regulation of ion channels by integrins. *Int Rev Cell Mol Biol* 279: 135-139, 2010.
12. **Beech DJ**. Ion channel switching and activation in smooth-muscle cells of occlusive vascular diseases. *Biochem Soc Trans* 35: 890–894, 2007.
13. **Beeton C**, **Pennington MW**, **Norton RS**. Analogs of the sea anemone potassium channel blocker ShK for the treatment of autoimmune diseases. *Inflamm Allergy Drug Targets* 10: 313–21, 2011.

14. **Beeton C**, **Wulff H**, **Standifer NE**, **Azam P**, **Mullen KM**, **Pennington MW**, **Kolski-Andreaco A**, **Wei E**, **Grino A**, **Counts DR**, **Wang PH**, **LeeHealey CJ**, **S Andrews B**, **Sankaranarayanan A**, **Homerick D**, **Roeck WW**, **Tehranzadeh J**, **Stanhope KL**, **Zimin P**, **Havel PJ**, **Griffey S**, **Knaus H-G**, **Nepom GT**, **Gutman G a**, **Calabresi PA**, **Chandy KG**. Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. *Proc Natl Acad Sci U S A* 103: 17414–17419, 2006.

15. **Di Bello IC**, **Dawson MR**, **Levine JM**, **Reynolds R**. Generation of oligodendroglial progenitors in acute inflammatory demyelinating lesions of the rat brain stem is associated with demyelination rather than inflammation. *J Neurocytol* 28: 365–81.

16. **Bezanilla F**. The voltage sensor in voltage-dependent ion channels. *Physiol Rev* 80: 555–592, 2000.

17. **Bezanilla F**. How membrane proteins sense voltage. *Nat Rev Mol Cell Biol* 9: 323–332, 2008.

18. **Binggeli R**, **Weinstein RC**. Membrane potentials and sodium channels: hypotheses for growth regulation and cancer formation based on changes in sodium channels and gap junctions. *J Theor Biol* 123: 377–401, 1986.

19. **Blackiston DJ, McLaughlin KA, Levin M**. Bioelectric controls of cell proliferation: Ion channels, membrane voltage and the cell cycle. *Cell Cycle* 8: 3527-36, 2009.

20. **Bock J**, **Szabó I**, **Gamper N**, **Adams C**, **Gulbins E**. Ceramide inhibits the potassium channel Kv1.3 by the formation of membrane platforms. *Biochem Biophys Res Commun* 305: 890–897, 2003.

21. **Bohuslavizki KH**, **Hänsel W**, **Kneip A**, **Koppenhöfer E**, **Niemöller E**, **Sanmann K**. Mode of action of psoralens, benzofurans, acridinons, and coumarins on the ionic currents in intact myelinated nerve fibres and its significance in demyelinating diseases. *Gen Physiol Biophys* 13: 309–28, 1994.

22. **Bonnet S**, **Archer SL**, **Allalunis-Turner J**, **Haromy A**, **Beaulieu C**, **Thompson R**, **Lee CT**, **Lopaschuk GD**, **Puttagunta L**, **Bonnet S**, **Harry G**, **Hashimoto K**, **Porter CJ**, **Andrade MA**, **Thebaud B**, **Michelakis ED**. A Mitochondria-K+ Channel Axis Is Suppressed in Cancer and Its Normalization Promotes Apoptosis and Inhibits Cancer Growth. *Cancer Cell* 11: 37–51, 2007.

23. **Cahalan MD**, **Chandy KG**. The functional network of ion channels in T lymphocytes. *Immunol Rev* 231: 59–87, 2009.

24. **Cahalan MD**, **Chandy KG**, **DeCoursey TE**, **Gupta S**. A voltage-gated potassium channel in human T lymphocytes. *J Physiol* 358: 197–237, 1985.

25. **Cahalan MD**, **Chandy KG**, **DeCoursey TE**, **Gupta S**. A voltage-gated potassium channel in human T lymphocytes. *J Physiol* 358: 197–237, 1985.

26. **Cahalan MD**, **Zhang SL**, **Yeromin A V**, **Ohlsen K**, **Roos J**, **Stauderman KA**. Molecular basis of the CRAC channel. *Cell Calcium* 42: 133–44, 2007.

27. **Castaneda O**, **Sotolongo V**, **Amor AM**, **Stocklin R**, **Anderson AJ**, **Harvey AL**, **Engstrom A**, **Wernstedt C**, **Karlsson E**. Characterization of a potassium channel toxin from the Caribbean Sea anemone *Stichodactyla helianthus*. *Toxicon* 33: 603–613, 1995.

28. **Chandy K**. Simplified gene nomenclature. *Nature* 352: 26–26, 1991.

29. **Chandy KG**, **Norton RS**. Peptide blockers of K v 1.3 channels in T cells as therapeutics for autoimmune disease. *Curr Opin Chem Biol* 38: 97–107, 2017.

30. **Chandy KG**, **Williams CB**, **Spencer RH**, **Aguilar BA**, **Ghanshani S**, **Tempel BL**, **Gutman GA**. A family of three mouse potassium channel genes with intronless coding regions. *Science* 247: 973–5, 1990.

31. **Chandy KG**, **Wulff H**, **Beeton C**, **Pennington M**, **Gutman GA**, **Cahalan MD**. K+ channels as targets for specific immunomodulation. *Trends Pharmacol Sci* 25: 280–289, 2004.

32. **Charolidi N**, **Schilling T**, **Eder C**. Microglial Kv1.3 Channels and P2Y12 Receptors Differentially Regulate Cytokine and Chemokine Release from Brain Slices of Young Adult and Aged Mice. *PLoS One* 10: e0128463, 2015.

33. **Cheng L**, **Yung A**, **Covarrubias M**, **Radice GL**. Cortactin is required for N-cadherin regulation of Kv1.5 channel function. *J Biol Chem* 286: 20478–89, 2011.

34. **Cheong A**, **Dedman AM**, **Beech DJ**. Expression and function of native potassium channel (Kv??1) subunits in terminal arterioles of rabbit. *J Physiol* 534: 691–700, 2001.

35. **Cheong A**, **Li J**, **Sukumar P**, **Kumar B**, **Zeng F**, **Riches K**, **Munsch C**, **Wood IC**, **Porter KE**, **Beech DJ**. Potent suppression of vascular smooth muscle cell migration and human neointimal hyperplasia by KV1.3 channel blockers. *Cardiovasc Res* 89: 282–289, 2011.

36. **Chittajallu R**, **Aguirre AA**, **Gallo V**. Downregulation of Platelet-Derived Growth Factor-α Receptor-Mediated Tyrosine Kinase Activity as a Cellular Mechanism for K^+-Channel Regulation during Oligodendrocyte Development In Situ. *Situ* 25: 8601–8610, 2005.

37. **Chittajallu R**, **Chen Y**, **Wang H**, **Yuan X**, **Ghiani CA**, **Heckman T**, **McBain CJ**, **Gallo V**. Regulation of Kv1 subunit expression in oligodendrocyte progenitor cells and their role in G1/S phase progression of the cell cycle. *Proc Natl Acad Sci U S A* 99: 2350–5, 2002.

38. **Choi KL**, **Aldrich RW**, **Yellen G**. Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated K+ channels. *Proc Natl Acad Sci U S A* 88: 5092–5, 1991.

39. **Chung I**, **Schlichter LC**. Native Kv1.3 channels are upregulated by protein kinase C. *J Membr Biol* 156: 73–85, 1997.

40. **Cidad P**, **Jiménez-Pérez L**, **García-Arribas D**, **Miguel-Velado E**, **Tajada S**, **Ruiz-Mcdavitt C**, **López-López JR**, **Pérez-García MT**. Kv1.3 channels can modulate cell proliferation during phenotypic switch by an ion-flux independent mechanism. *Arterioscler Thromb Vasc Biol* 32: 1299–307, 2012.

41. **Cidad P**, **Miguel-Velado E**, **Ruiz-McDavitt C**, **Alonso E**, **Jiménez-Pérez L**, **Asuaje A**, **Carmona Y**, **García-Arribas D**, **López J**, **Marroquín Y**, **Fernández M**, **Roqué M**, **Pérez-García MT**, **López-López JR**. Kv1.3 channels modulate human vascular smooth muscle cells proliferation independently of mTOR signaling pathway. *Pflügers Arch - Eur J Physiol* 467: 1711–1722, 2015.

42. **Cidad P**, **Moreno-Domínguez A**, **Novensá L**, **Roqué M**, **Barquín L**, **Heras M**, **Pérez-García MT**, **López-López JR**. Characterization of ion channels involved in the proliferative response of femoral artery smooth muscle cells. *Arterioscler Thromb Vasc Biol* 30: 1203–11, 2010.

43. **Cidad P**, **Novensà L**, **Garabito M**, **Batlle M**, **Dantas AP**, **Heras M**, **López-López JR**, **Pérez-Garc'ia MT**, **Roqué M**. K+ Channels Expression in Hypertension After Arterial Injury, and Effect of Selective Kv1.3 Blockade with PAP-1 on Intimal Hyperplasia Formation. *Cardiovasc Drugs Ther* 28: 501–511, 2014.

44. **Coetzee W a**, **Amarillo Y**, **Chiu J**, **Chow a**, **Lau D**, **McCormack T**, **Moreno H**, **Nadal MS**, **Ozaita a**, **Pountney D**, **Saganich M**, **Vega-Saenz de Miera E**, **Rudy B**. Molecular diversity of K+ channels. *Ann N Y Acad Sci* 868: 233–285, 1999.

45. **Colley BS**, **Biju KC**, **Visegrady A**, **Campbell S**, **Fadool DA**. Neurotrophin B receptor kinase increases Kv subfamily member 1.3 (Kv1.3) ion channel half-life and surface expression. *Neuroscience* 144: 531–546, 2007.

46. **Colley BS**, **Cavallin MA**, **Biju K**, **Marks DR**, **Fadool DA**. Brain-derived neurotrophic factor modulation of Kv1.3 channel is disregulated by adaptor proteins Grb10 and nShc. *BMC Neurosci* 10: 8, 2009.

47. **Cook KK**, **Fadool DA**. Two adaptor proteins differentially modulate the phosphorylation and biophysics of Kv1.3 ion channel by SRC kinase. *J Biol Chem* 277: 13268–80, 2002.

48. **Daly RJ**. Cortactin signalling and dynamic actin networks. *Biochem J* 382: 13–25, 2004.

49. **DeCoursey TE**, **Chandy KG**, **Gupta S**, **Cahalan MD**. Voltage-gated K+ channels in human T lymphocytes: a role in mitogenesis? *Nature* 307: 465–8.

50. **DeCoursey TE**, **Chandy KG**, **Gupta S**, **Cahalan MD**. Voltage-gated K+ channels in human T lymphocytes: a role in mitogenesis? *Nature* 307: 465–468, 1984.

51. **DeCoursey TE**, **Chandy KG**, **Gupta S**, **Cahalan MD.** Two types of potassium channels in murine T lymphocytes. 89: 379–404, 1987.

52. **Doyle DA**, **Cabral JM**, **Pfuetzner RA**, **Kuo A**, **Gulbis JM**, **Cohen SL**, **Chait BT**,

MacKinnon R. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. *Science* 280: 69–77, 1998.

53. **Dubois J-M**, **Rouzaire-Dubois B**. The influence of cell volume changes on tumour cell proliferation. *Eur Biophys J* 33: 227–232, 2004.

54. **Eil R**, **Vodnala SK**, **Clever D**, **Klebanoff CA**, **Sukumar M**, **Pan JH**, **Palmer DC**, **Gros A**, **Yamamoto TN**, **Patel SJ**, **Guittard GC**, **Yu Z**, **Carbonaro V**, **Okkenhaug K**, **Schrump DS**, **Linehan WM**, **Roychoudhuri R**, **Restifo NP**. Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature* 537: 539–543, 2016.

55. **Evans J V**, **Kelley LC**, **Hayes KE**, **Ammer AG**, **Martin KH**, **Weed SA**. Further insights into cortactin conformational regulation. *Bioarchitecture* 1: 21–23, 2011.

56. **Fadool DA**, **Holmes TC**, **Berman K**, **Dagan D**, **Levitan IB**. Tyrosine Phosphorylation Modulates Current Amplitude and Kinetics of a Neuronal Voltage-Gated Potassium Channel. *J Neurophysiol* 78, 1997.

57. **Fadool DA**, **Tucker K**, **Perkins R**, **Fasciani G**, **Thompson RN**, **Parsons AD**, **Overton JM**, **Koni PA**, **Flavell RA**, **Kaczmarek LK**. Kv1.3 channel gene-targeted deletion produces "Super-Smeller Mice" with altered glomeruli, interacting scaffolding proteins, and biophysics. *Neuron* 41: 389–404, 2004.

58. **Fan L**, **Di Ciano-Oliveira C**, **Weed SA**, **Craig AWB**, **Greer PA**, **Rotstein OD**, **Kapus A**. Actin depolymerization-induced tyrosine phosphorylation of cortactin: the role of Fer kinase. *Biochem J* 380: 581–91, 2004.

59. **Feske S**. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca2+ entry in the immune system and beyond. *Immunol Rev* 231: 189–209, 2009.

60. **Gehrmann J**, **Matsumoto Y**, **Kreutzberg GW**. Microglia: intrinsic immuneffector cell of the brain. *Brain Res Brain Res Rev* 20: 269–87, 1995.

61. **Ginsberg MH**. Integrin activation. *BMB Rep* 47: 655–9, 2014.

62. **Ginsberg MH**, **Partridge A**, **Shattil SJ**. Integrin regulation. *Curr Opin Cell Biol* 17: 509–516, 2005.

63. **Grigoryan G**, **Moore DT**, **DeGrado WF**. Transmembrane communication: general principles and lessons from the structure and function of the M2 proton channel, K^+ channels, and integrin receptors. *Annu Rev Biochem* 80: 211–37, 2011.

64. **Grissmer S**, **Dethlefst B**, **Wasmutht JJ**, **Goldin AL**, **Gutman GA**, **Cahalan MD**, **Chandy KG**. Expression and chromosomal localization of a lymphocyte K + channel gene. *Proc Natl Acad Sci* 87: 9411–9415, 1990.

65. **Grunnet M**, **Rasmussen HB**, **Hay-Schmidt A**, **Rosenstierne M**, **Klaerke DA**, **Olesen S-P**, **Jespersen T**. KCNE4 Is an Inhibitory Subunit to Kv1.1 and Kv1.3 Potassium

Channels. *Biophys J* 85: 1525–1537, 2003.

66. **Gulbis JM**, **Mann S**, **MacKinnon R**, **Quiocho FA**, **Jan LY**, **Trimmer JS**, **Dolly JO**, **MacKinnon R**, **Nilges M**, **Pannu NS**, **al. et**. Structure of a voltage-dependent K+ channel beta subunit. *Cell* 97: 943–52, 1999.

67. **Gutman GA**, **Chandy KG**, **Grissmer S**, **Lazdunski M**, **McKinnon D**, **Pardo LA**, **Robertson GA**, **Rudy B**, **Sanguinetti MC**, **Stühmer W**, **Wang X**. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* 57: 473–508, 2005.

68. **Hajdu P**, **Martin G V.**, **Chimote A a.**, **Szilagyi O**, **Takimoto K**, **Conforti L**. The C-terminus SH3-binding domain of Kv1.3 is required for the actin-mediated immobilization of the channel via cortactin. *Mol Biol Cell* 26: 1640–1651, 2015.

69. **Hanada T**, **Lin L**, **Chandy KG**, **Oh SS**, **Chishti AH**. Human Homologue of the Drosophila Discs Large Tumor Suppressor Binds to p56 lck Tyrosine Kinase and Shaker Type Kv1.3 Potassium Channel in T Lymphocytes. *J Biol Chem* 272: 26899–26904, 1997.

70. **Head BP**, **Patel HH**, **Insel PA**. Interaction of membrane/lipid rafts with the cytoskeleton: Impact on signaling and function: Membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling. *Biochim Biophys Acta - Biomembr* 1838: 532–545, 2014.

71. **Hegle AP**, **Marble DD**, **Wilson GF**. A voltage-driven switch for ion-independent signaling by ether-à-go-go K+ channels. *Proc Natl Acad Sci U S A* 103: 2886–91, 2006.

72. **Herrmann S**, **Ninkovic M**, **Kohl T**, **Lörinczi É**, **Pardo LA**. Cortactin controls surface expression of the voltage-gated potassium channel K(V)10.1. *J Biol Chem* 287: 44151–63, 2012.

73. **Ho CS**, **Grange RW**, **Joho RH**. Pleiotropic effects of a disrupted K+ channel gene: reduced body weight, impaired motor skill and muscle contraction, but no seizures. *Proc Natl Acad Sci U S A* 94: 1533–8, 1997.

74. **Hoffmann EK**, **Lambert IH**, **Pedersen SF**. Physiology of cell volume regulation in vertebrates. *Physiol Rev* 89: 193–277, 2009.

75. **Holmes T**, **Fadool D**, **Levitan I**. Tyrosine phosphorylation of the Kv1.3 potassium channel. *J Neurosci* 16: 1581-90, 1996.

76. **Hoshi T**, **Zagotta WN**, **Aldrich RW**. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science* 250: 533–8, 1990.

77. **Jiang Y**, **Ruta V**, **Chen J**, **Lee A**, **MacKinnon R**, **Cadene M**, **Chalt BT**. X-ray structure of a voltage-dependent {K}^+ channel. *Nature* 423: 42–48, 2003.

78. **Jiménez-Pérez L**, **Cidad P**, **Álvarez-Miguel I**, **Santos-Hipólito A**, **Torres-Merino R**, **Alonso E**, **de la Fuente MÁ**, **López-López JR**, **Pérez-García MT**. Molecular

Determinants of Kv1.3 Potassium Channels-Induced Proliferation. *J Biol Chem* 291: 1–13, 2015.

79. **K??hler R**, **Wulff H**, **Eichler I**, **Kneifel M**, **Neumann D**, **Knorr A**, **Grgic I**, **K??mpfe D**, **Si H**, **Wibawa J**, **Real R**, **Borner K**, **Brakemeier S**, **Orzechowski HD**, **Reusch HP**, **Paul M**, **Chandy KG**, **Hoyer J**. Blockade of the intermediate-conductance calcium-activated potassium channel as a new therapeutic strategy for restenosis. *Circulation* 108: 1119–1125, 2003.

80. **Kaczmarek LK**. Non-conducting functions of voltage-gated ion channels. *Nat Rev Neurosci* 7: 761–771, 2006.

81. **Kamb A**, **Tseng-Crank J**, **Tanouye MA**. Multiple products of the drosophila Shaker gene may contribute to potassium channel diversity. *Neuron* 1: 421–430, 1988.

82. **Kettenmann H**, **Banati R**, **Walz W**. Electrophysiological behavior of microglia. *Glia* 7: 93–101, 1993.

83. **Kettenmann H**, **Hanisch U-K**, **Noda M**, **Verkhratsky A**. Physiology of Microglia. *Physiol Rev* 91: 461-553, 2011.

84. **Kim E**, **Niethammer M**, **Rothschild A**, **Nung Jan Y**, **Sheng M**. Clustering of Shaker-type K+ channels by interaction with a family of membrane-associated guanylate kinases. *Nature* 378: 85–88, 1995.

85. **Kirkbride KC**, **Sung BH**, **Sinha S**, **Weaver AM**. Cortactin: a multifunctional regulator of cellular invasiveness. *Cell Adh Migr* 5: 187–98, 2011.

86. **Koo GC**, **Blake JT**, **Talento A**, **Nguyen M**, **Lin S**, **Sirotina A**, **Shah K**, **Mulvany K**, **Hora D**, **Cunningham P**, **Wunderler DL**, **McManus OB**, **Slaughter R**, **Bugianesi R**, **Felix J**, **Garcia M**, **Williamson J**, **Kaczorowski G**, **Sigal NH**, **Springer MS**, **Feeney W**. Blockade of the voltage-gated potassium channel Kv1.3 inhibits immune responses in vivo. *J Immunol* 158, 1997.

87. **Koshy S**, **Wu D**, **Hu X**, **Tajhya RB**, **Huq R**, **Khan FS**, **Pennington MW**, **Wulff H**, **Yotnda P**, **Beeton C**. Blocking KCa3.1 Channels Increases Tumor Cell Killing by a Subpopulation of Human Natural Killer Lymphocytes. *PLoS One* 8: 1–11, 2013.

88. **Kotecha SA**, **Schlichter LC**. A Kv1.5 to Kv1.3 switch in endogenous hippocampal microglia and a role in proliferation. *J Neurosci* 19: 10680–10693, 1999.

89. **Kumar B**, **Dreja K**, **Shah SS**, **Cheong A**, **Xu SZ**, **Sukumar P**, **Naylor J**, **Forte A**, **Cipollaro M**, **McHugh D**, **Kingston PA**, **Heagerty AM**, **Munsch CM**, **Bergdahl A**, **Hultg??rdh-Nilsson A**, **Gomez MF**, **Porter KE**, **Hellstrand P**, **Beech DJ**. Upregulated TRPC1 channel in vascular injury in vivo and its role in human neointimal hyperplasia. *Circ Res* 98: 557–563, 2006.

90. **Kunzelmann K**. Ion channels and cancer. *J Membr Biol* 205: 159–173, 2005.

91. **Kuras Z**, **Kucher V**, **Gordon SM**, **Neumeier L**, **Chimote AA**, **Filipovich AH**, **Conforti L**. Modulation of Kv1.3 channels by protein kinase A I in T lymphocytes is mediated by the disc large 1-tyrosine kinase Lck complex. *Am J Physiol Cell Physiol* 302: C1504-12, 2012.

92. **Lang F**, **Föller M**, **Lang K**, **Lang P**, **Ritter M**, **Vereninov A**, **Szabo I**, **M. Huber S**, **Gulbins E**. Cell Volume Regulatory Ion Channels in Cell Proliferation and Cell Death. *Methods Enzymol* 428: 209–225, 2007.

93. **Lastraioli E**, **Iorio J**, **Arcangeli A**. Ion channel expression as promising cancer biomarker. *Biochim Biophys Acta* 8571922: 2685–2702, 2014.

94. **Leanza L**, **Biasutto L**, **Managò A**, **Gulbins E**, **Zoratti M**, **Szabò I**. Intracellular ion channels and cancer. *Front Physiol* 4: 227, 2013.

95. **Lee A**, **Fakler B**, **Kaczmarek LK**, **Isom LL**. More Than a Pore: Ion Channel Signaling Complexes. *J Neurosci* 34: 15159–15169, 2014.

96. **Levin M**. Molecular bioelectricity in developmental biology: New tools and recent discoveries: Control of cell behavior and pattern formation by transmembrane potential gradients. *BioEssays* 34: 205–217, 2012.

97. **Levite BM**, **Cahalon L**, **Peretz A**, **Hershkoviz R**, **Sobko A**, **Ariel A**, **Desai R**, **Attali B**, **Lider O**. Extracellular K^+ and Opening of Voltage-gated Potassium Channels Activate T Cell Integrin Function : Physical and Functional Association between Kv1.3 Channels and beta 1 Integrins. *J Exp Med* 191: 1167-76, 2000.

98. **Levy DI**, **Deutsch C**. Recovery from C-type inactivation is modulated by extracellular potassium. *Biophys J* 70: 798–805, 1996.

99. **Lewis RS**, **Cahalan MD**. Subset-specific expression of potassium channels in developing murine T lymphocytes. *Science* 239: 771–5, 1988.

100. **Liu S**, **Rasmusson RL**. Models of potassium channel inactivation: implications for open channel block. *Can J Cardiol* 14: 101–7, 1998.

101. **Long SB, Campbell EB, Mackinnon R.** Crystal Structure of a Mammalian Voltage-Dependent Shaker Family K+ Channel. *Science (80-)* 309: 897–903, 2005.

102. **Long SB**, **Campbell EB**, **Mackinnon R**. Crystal Structure of a Mammalian Voltage-Dependent Shaker Family K+ Channel. *Science* 309: 897–903, 2005.

103. **López-Barneo J**, **Hoshi T**, **Heinemann SH**, **Aldrich RW**. Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. *Receptors Channels* 1: 61–71, 1993.

104. **Macfarlane SN**, **Sontheimer H**. Electrophysiological Changes that Acompany Reactive Gliosis In Vitro. *In Vitro. J Neurosci* 17: 7316–7329, 1997.

105. **MacFarlane SN**, **Sontheimer H**. Changes in ion channel expression accompany cell

cycle progression of spinal cord astrocytes. *Glia* 30: 39–48, 2000.

106. **Mahnke YD**, **Brodie TM**, **Sallusto F**, **Roederer M**, **Lugli E**. The who's who of T-cell differentiation: human memory T-cell subsets. *Eur J Immunol* 43: 2797–809, 2013.

107. **Marks DR**, **Fadool DA**. Post-synaptic density perturbs insulin-induced Kv1.3 channel modulation via a clustering mechanism involving the SH $_3$ domain. *J Neurochem* 103: 1608–1627, 2007.

108. **Martinez-Marmol R**, **Perez-Verdaguer M**, **Roig SR**, **Vallejo-Gracia A**, **Gotsi P**, **Serrano-Albarras A**, **Bahamonde MI**, **Ferrer-Montiel A**, **Fernandez-Ballester G**, **Comes N**, **Felipe A**. A non-canonical di-acidic signal at the C-terminus of Kv1.3 determines anterograde trafficking and surface expression. *J Cell Sci* 126: 5681–5691, 2013.

109. **McCormack T**, **McCormack K**, **Nadal MS**, **Vieira E**, **Ozaita a**, **Rudy B**. The effects of Shaker beta-subunits on the human lymphocyte K+ channel Kv1.3. *J Biol Chem* 274: 20123–20126, 1999.

110. **Miguel-Velado E**, **Pérez-Carretero FD**, **Colinas O**, **Cidad P**, **Heras M**, **López-López JR**, **Pérez-García MT**. Cell cycle-dependent expression of Kv3.4 channels modulates proliferation of human uterine artery smooth muscle cells. *Cardiovasc Res* 86: 383–91, 2010.

111. **Millership JE**, **Devor DC**, **Hamilton KL**, **Balut CM**, **Bruce JIE**, **Fearon IM**. Calcium-activated K+ channels increase cell proliferation independent of K+ conductance. *Am J Physiol Cell Physiol* 300: C792–C802, 2011.

112. **Newby AC**, **Zaltsman AB**. Molecular mechanisms in intimal hyperplasia. *J Pathol* 190: 300–309, 2000.

113. **Newell EW**, **Schlichter LC**. Integration of K+ and Cl- currents regulate steady-state and dynamic membrane potentials in cultured rat microglia. *J Physiol* 567: 869–890, 2005.

114. **Neylon CB**. Potassium channels and vascular proliferation. *Vasc Pharmacol* 38: 35–41, 2002.

115. **Nicolaou SA**, **Neumeier L**, **Steckly A**, **Kucher V**, **Takimoto K**, **Conforti L**. Localization of Kv1.3 channels in the immunological synapse modulates the calcium response to antigen stimulation in T lymphocytes. *J Immunol* 183: 6296–302, 2009.

116. **Nicolaou SA**, **Neumeier L**, **Takimoto K**, **Lee SM**, **Duncan HJ**, **Kant SK**, **Mongey AB**, **Filipovich AH**, **Conforti L**. Differential calcium signaling and Kv1.3 trafficking to the immunological synapse in systemic lupus erythematosus. *Cell Calcium* 47: 19–28, 2010.

117. **Nörenberg W**, **Gebicke-Haerter PJ**, **Illes P**. Inflammatory stimuli induce a new K+ outward current in cultured rat microglia. *Neurosci Lett* 147: 171–174, 1992.

118. **Ouadid-Ahidouch H**, **Ahidouch A**. K+ channels and cell cycle progression in tumor

cells. *Front Physiol* 4:220: 1–8, 2013.

119. **Owens GK**, **Kumar MS**, **Wamhoff BR**. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* 84: 767–801, 2004.

120. **Pahapill PA**, **Schlichter LC**. Modulation of potassium channels in intact human T lymphocytes. *J Physiol* 445: 407–30, 1992.

121. **Pannasch U**, **Farber K**, **Nolte C**, **Blonski M**, **Yan Chiu S**, **Messing A**, **Kettenmann H**, **Färber K**, **Nolte C**, **Blonski M**, **Yan Chiu S**, **Messing A**, **Kettenmann H**. The potassium channels Kv1.5 and Kv1.3 modulate distinct functions of microglia. *Mol Cell Neurosci* 33: 401–11, 2006.

122. **Panyi G**. Biophysical and pharmacological aspects of K+ channels in T lymphocytes. *Eur Biophys J* 34: 515–529, 2005.

123. **Panyi G**, **Sheng Z**, **Deutsch C**. C-type inactivation of a voltage-gated K+ channel occurs by a cooperative mechanism. *Biophys J* 69: 896–903, 1995.

124. **Panyi G**, **Vamosi G**, **Bacso Z**, **Bagdany M**, **Bodnar A**, **Varga Z**, **Gaspar R**, **Matyus L**, **Damjanovich S**. Kv1.3 potassium channels are localized in the immunological synapse formed between cytotoxic and target cells. *Proc Natl Acad Sci* 101: 1285–1290, 2004.

125. **Papazian DM**, **Schwarz TL**, **Tempel BL**, **Jan YN**, **Jan LY**. Cloning of genomic and complementary DNA from Shaker, a putative potassium channel gene from Drosophila. *Science* 237: 749–53, 1987.

126. **Pardo L.** Voltage-gated potassium channels in cell proliferation. *Physiology* 19: 285–92, 2004.

127. **Pathak M**, **Kurtz L**, **Tombola F**, **Isacoff E**. The cooperative voltage sensor motion that gates a potassium channel. *J Gen Physiol* 125: 57–69, 2005.

128. **Pegg AE**. Functions of Polyamines in Mammals. *J Biol Chem* 291: 14904–12, 2016.

129. **Penna A**, **Demuro A**, **Yeromin A V**, **Zhang SL**, **Safrina O**, **Parker I**, **Cahalan MD**. The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature* 456: 116–20, 2008.

130. **Pennington M**, **Chang S**, **Chauhan S**, **Huq R**, **Tajhya R**, **Chhabra S**, **Norton R**, **Beeton C**. Development of Highly Selective Kv1.3-Blocking Peptides Based on the Sea Anemone Peptide ShK. *Mar Drugs* 13: 529–542, 2015.

131. **Pérez-verdaguer M**, **Capera J**, **Martínez-mármol R**, **Camps M**. Caveolin interaction governs Kv1.3 lipid raft targeting. *Sci Rep* 6: 22453, 2016.

132. **Perozo E**, **MacKinnon R**, **Bezanilla F**, **Stefani E**. Gating currents from a nonconducting mutant reveal open-closed conformations in Shaker K+ channels. *Neuron* 11: 353–8, 1993.

133. **Plane F**, **Johnson R**, **Kerr P**, **Wiehler W**, **Thorneloe K**, **Ishii K**, **Chen T**, **Cole W**. Heteromultimeric Kv1 channels contribute to myogenic control of arterial diameter. *Circ Res* 96: 216–224, 2005.

134. **Pongs O**, **Kecskemethy N**, **Müller R**, **Krah-Jentgens I**, **Baumann A**, **Kiltz HH**, **Canal I**, **Llamazares S**, **Ferrus A**. Shaker encodes a family of putative potassium channel proteins in the nervous system of Drosophila. *EMBO J* 7: 1087–96, 1988.

135. **Pongs O**, **Schwarz JR**. Ancillary Subunits Associated With Voltage-Dependent K+ Channels. *Physiol Rev* 90: 755-96, 2010.

136. **Quintana A**, **Pasche M**, **Junker C**, **Al-Ansary D**, **Rieger H**, **Kummerow C**, **Nuñez L**, **Villalobos C**, **Meraner P**, **Becherer U**, **Rettig J**, **Niemeyer BA**, **Hoth M**. Calcium microdomains at the immunological synapse: how ORAI channels, mitochondria and calcium pumps generate local calcium signals for efficient T-cell activation. *EMBO J* 30: 3895–3912, 2011.

137. **Rangaraju S**, **Gearing M**, **Jin L-W**, **Levey A**. Potassium channel Kv1.3 is highly expressed by microglia in human Alzheimer's disease. *J Alzheimers Dis* 44: 797–808, 2015.

138. **Rasmusson RL**, **Morales MJ**, **Wang S**, **Liu S**, **Campbell DL**, **Brahmajothi M V.**, **Strauss HC**. Inactivation of Voltage-Gated Cardiac K+ Channels. *Circ Res* 82: 739-50, 1998.

139. **Runnels LW**, **Yue L**, **Clapham DE**. TRP-PLIK, a bifunctional protein with kinase and ion channel activities. *Science* 291: 1043–7, 2001.

140. **Schlichter LC**, **Sakellaropoulos G**, **Ballyk B**, **Pennefather PS**, **Phipps DJ**. Properties of K+ and Cl- channels and their involvement in proliferation of rat microglial cells. *Glia* 17: 225–36, 1996.

141. **Schmitz A**, **Sankaranarayanan A**, **Azam P**, **Schmidt-Lassen K**, **Homerick D**, **Hansel W**, **Wulff H**. Design of PAP-1, a selective small molecule Kv1.3 blocker, for the suppression of effector memory T cells in autoimmune diseases. *Mol Pharmacol* 68: 1254–1270, 2005.

142. **Schwarz TL**, **Tempel BL**, **Papazian DM**, **Jan YN**, **Jan LY**. Multiple potassium–channel components are produced by alternative splicing at the Shaker locus in Drosophila. *Nature* 331: 137–142, 1988.

143. **Shen NV**, **Pfaffinger PJ**. Molecular recognition and assembly sequences involved in the subfamily-specific assembly of voltage-gated K+ channel subunit proteins. *Neuron* 14: 625–633, 1995.

144. **Sobko A**, **Peretz A**, **Shirihai O**, **Etkin S**, **Cherepanova V**, **Dagan D**, **Attali B**. Heteromultimeric Delayed-Rectifier K+ Channels in Schwann Cells: Developmental Expression and Role in Cell Proliferation. *J Neurosci* 18: 10398-408, 1998.

145. **Solé L**, **Roig SR**, **Vallejo-Gracia A**, **Serrano-Albarrás A**, **Martínez-Mármol R**, **Tamkun MM**, **Felipe A**. The C-terminal domain of Kv1.3 regulates functional interactions with the KCNE4 subunit. *J Cell Sci* 129: 4265–4277, 2016.

146. **Solé L**, **Roura-Ferrer M**, **Pérez-Verdaguer M**, **Oliveras A**, **Calvo M**, **Fernández-Fernández JM**, **Felipe A**. KCNE4 suppresses Kv1.3 currents by modulating trafficking, surface expression and channel gating. *J Cell Sci* 122: 3738-48, 2009.

147. **Somodi S**, **Varga Z**, **Hajdu P**, **Starkus JG**, **Levy DI**, **Gáspár R**, **Panyi G**. pH-dependent modulation of Kv1.3 inactivation: role of His399. *AJP Cell Physiol* 287: C1067–C1076, 2004.

148. **Spear JM**, **Koborssy D Al**, **Schwartz AB**, **Johnson AJ**, **Audhya A**, **Fadool DA**, **Stagg SM**. Kv1.3 contains an alternative C-terminal ER exit motif and is recruited into COPII vesicles by Sec24a. *BMC Biochem* 16: 16, 2015.

149. **Stebbing MJ**, **Cottee JM**, **Rana I**. The role of ion channels in microglial activation and proliferation - a complex interplay between ligand-gated ion channels, K+ channels, and intracellular Ca2+. *Front Immunol* 6: 1–7, 2015.

150. **Strang C**, **Cushman SJ**, **DeRubeis D**, **Peterson D**, **Pfaffinger PJ**. A Central Role for the T1 Domain in Voltage-gated Potassium Channel Formation and Function. *J Biol Chem* 276: 28493–28502, 2001.

151. **Stühmer W**, **Ruppersberg JP**, **Schröter KH**, **Sakmann B**, **Stocker M**, **Giese KP**, **Perschke A**, **Baumann A**, **Pongs O**. Molecular basis of functional diversity of voltage-gated potassium channels in mammalian brain. *EMBO J* 8: 3235–44, 1989.

152. **Swanson R**, **Marshall J**, **Smith JS**, **Williams JB**, **Boyle MB**, **Folander K**, **Luneau CJ**, **Antanavage J**, **Oliva C**, **Buhrow SA**, **Bennet C**, **Stein RB**, **Kaczmarek LK**. Cloning and expression of cDNA and genomic clones encoding three delayed rectifier potassium channels in rat brain. *Neuron* 4: 929–939, 1990.

153. **Szabò I**, **Bock J**, **Jekle A**, **Soddemann M**, **Adams C**, **Lang F**, **Zoratti M**, **Gulbins E**. A novel potassium channel in lymphocyte mitochondria. *J Biol Chem* 280: 12790–12798, 2005.

154. **Szigligeti P**, **Neumeier L**, **Duke E**, **Chougnet C**, **Takimoto K**, **Lee SM**, **Filipovich AH**, **Conforti L**. Signalling during hypoxia in human T lymphocytes--critical role of the src protein tyrosine kinase p56Lck in the O2 sensitivity of Kv1.3 channels. *J Physiol* 573: 357–370, 2006.

155. **Szilágyi O**, **Boratkó A**, **Panyi G**, **Hajdu P**. The role of PSD-95 in the rearrangement of Kv1.3 channels to the immunological synapse. *Pflügers Arch - Eur J Physiol* 465: 1341–1353, 2013.

156. **Tarcha EJ**, **Chi V**, **Munoz-Elias EJ**, **Bailey D**, **Londono LM**, **Upadhyay SK**, **Norton K**, **Banks A**, **Tjong I**, **Nguyen H**, **Hu X**, **Ruppert GW**, **Boley SE**, **Slauter R**, **Sams J**,

Knapp B, **Kentala D**, **Hansen Z**, **Pennington MW**, **Beeton C**, **Chandy KG**, **Iadonato SP**. Durable Pharmacological Responses from the Peptide ShK-186, a Specific Kv1.3 Channel Inhibitor That Suppresses T Cell Mediators of Autoimmune Disease. *J Pharmacol Exp Ther* 342: 642–653, 2012.

157. **Teisseyre A**, **Gąsiorowska J**, **Michalak K**. Voltage-Gated Potassium Channels Kv1.3 - Potentially New Molecular Target in Cancer Diagnostics and Therapy. *Adv Clin Exp Med* 24: 517–524, 2015.

158. **Urrego D**, **Tomczak AP**, **Zahed F**, **Stühmer W**, **Pardo LA**. Potassium channels in cell cycle and cell proliferation. *Philos Trans R Soc Lond B Biol Sci* 369: 20130094, 2014.

159. **Vautier FF**, **Belachew S**, **Chittajallu R**, **Gallo V**. Shaker-type potassium channel subunits differentially control oligodendrocyte progenitor proliferation. *Glia* 48: 337–345, 2004.

160. **Veh RW**, **Lichtinghagen R**, **Sewing S**, **Wunder F**, **Grumbach IM**, **Pongs O**. Immunohistochemical localization of five members of the Kv1 channel subunits: contrasting subcellular locations and neuron-specific co-localizations in rat brain. *Eur J Neurosci* 7: 2189–205, 1995.

161. **Vennekamp J**, **Wulff H**, **Beeton C**, **Calabresi PA**, **Grissmer S**, **Hansel W**, **Chandy KG**. Kv1.3-blocking 5-phenylalkoxypsoralens: a new class of immunomodulators. *Mol Pharmacol* 65: 1364–1374, 2004.

162. **Verheugen JA**, **Vijverberg HP**, **Oortgiesen M**, **Cahalan MD**. Voltage-gated and Ca(2+)-activated K+ channels in intact human T lymphocytes. Noninvasive measurements of membrane currents, membrane potential, and intracellular calcium. *J Gen Physiol* 105: 765–94, 1995.

163. **Vicente R**, **Escalada A**, **Coma M**, **Fuster G**, **Sánchez-Tilló E**, **López-Iglesias C**, **Soler C**, **Solsona C**, **Celada A**, **Felipe A**. Differential voltage-dependent K+ channel responses during proliferation and activation in macrophages. *J Biol Chem* 278: 46307–20, 2003.

164. **Vicente R**, **Villalonga N**, **Calvo M**, **Escalada A**, **Solsona C**, **Soler C**, **Tamkun MM**, **Felipe A**. Kv1.5 association modifies Kv1.3 traffic and membrane localization. *J Biol Chem* 283: 8756–8764, 2008.

165. **Villalonga N**, **David M**, **Bielanska J**, **Vicente R**, **Comes N**, **Valenzuela C**, **Felipe A**. Immunomodulation of voltage-dependent K+ channels in macrophages: molecular and biophysical consequences. *J Gen Physiol* 135: 135–47, 2010.

166. **Villalonga N**, **Escalada A**, **Vicente R**, **Sánchez-Tilló E**, **Celada A**, **Solsona C**, **Felipe A**. Kv1.3/Kv1.5 heteromeric channels compromise pharmacological responses in macrophages. *Biochem Biophys Res Commun* 352: 913–8, 2007.

167. **Wamhoff BR**, **Bowles DK**, **Owens GK**. Excitation-transcription coupling in arterial

smooth muscle. *Circ Res* 98: 868–878, 2006.

168. **Watanabe M**, **Toyama Y**, **Nishiyama A**. Differentiation of proliferated NG2-positive glial progenitor cells in a remyelinating lesion. *J Neurosci Res* 69: 826–36, 2002.

169. **Williams MR**, **Markey JC**, **Doczi MA**, **Morielli AD**. An essential role for cortactin in the modulation of the potassium channel Kv1.2. *Proc Natl Acad Sci U S A* 104: 17412–7, 2007.

170. **Wonderlin WF**, **Strobl JS**. Potassium channels, proliferation and G1 progression. *J Membr Biol* 154: 91–107, 1996.

171. **Wulff H**, **Calabresi PA**, **Allie R**, **Yun S**, **Pennington M**, **Beeton C**, **Chandy KG**. The voltage-gated Kv1 . 3 K + channel in effector memory T cells as new target for MS. *J Clin Invest* 111: 1703–1713, 2003.

172. **Wulff H**, **Knaus H-G**, **Pennington M**, **Chandy KG**. K+ channel expression during B cell differentiation: implications for immunomodulation and autoimmunity. *J Immunol* 173: 776–86, 2004.

173. **Wulff H**, **Zhorov BS**. K+ Channel Modulators for the Treatment of Neurological Disorders and Autoimmune Diseases. *Chem Rev* 108: 1744-73, 2008.

174. **Zhu J**, **Yan J**, **Thornhill WB**. The Kv1.3 potassium channel is localized to the cis-Golgi and Kv1.6 is localized to the endoplasmic reticulum in rat astrocytes. *FEBS J* 281: 3433–45, 2014.

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Figure legends

Figure 1.A. Schematic picture of human Kv1.3 α-subunit highlighting some important domains. The S4 segment sequence with the charged residues in red (16), a N-glycosylation site (174) and the pore region sequence with a tryptophan residue critical for ion permeation and the K+ selectivity filter highlighted (52, 132) are shown on top. The lower part shows the N- and C-terminal sequences with several well-characterized regions, including the SH3 domain, (107), the tetramerization T1 domain (150), the Kvβ association signature (135), and Caveolin binding domain (131) on the N-terminus, and the HRETE (148) or YMVIEE (108) forward trafficking motifs, the YS-segment (78) another SH3 binding domain and the PDZ binding domain (68, 107) on the C-terminus. The sequence of the S4-S5 linker (77) is also shown. The intracellular phosphorylatable residues are depicted as black dots. **B.** Schematic illustration of some of the described associations of Kv1.3 channels, including the cholesterol binding protein caveolin, and a heterodimeric integrin receptor at the cell membrane. Intracellular associated proteins include Kvβ subunits, PDZ proteins associating Kv1.3 with kinases, and cortactin binding F-actin. The focal adhesion complex links the cytoskeleton to integrin receptor. **C.** Functional domains of Kv1.3 channels. Kv1.3 channels are voltage sensors (sensor domain) that respond to changes in membrane potential with a conformational change. This conformational change activates K^+ permeability (pore domain) leading to changes in V_M. The cytoplasmic N- and C-terminal regions (signaling domain) can serve as docking sites for protein-protein interactions with other intracellular proteins that can activate different signaling pathways.

Figure 2. The membrane potential model of Kv1.3-induced proliferation. Activation of the immune synapse of a T-cell by an APC leads to PLC activation. PLC catalyzes the production of DAG (not shown) and IP3, which binds to its receptor on the ER, resulting in an initial small increased in cytoplasmic Ca^{2+}. To sustain T-cell activation, an additional Ca^{2+} influx is required. This influx is mediated by the voltage-independent Ca^{2+} channel Orai, which is activated by STIM1 upon depletion of Ca^{2+} from intracellular stores. This Ca^{2+} influx activates KCa3.1, and by depolarizing the cell also activates Kv1.3. These K^+ channels led to membrane hyperpolarization that sustains Ca^{2+} entry. The subsequent increase in $[Ca^{2+}]_i$ mediates NFAT, translocation to the nucleus through the activation of calcineurin phosphatase. The normal values of resting V_M and of intra- and extracellular concentrations of K^+ and Ca^{2+}, and their reversal potentials are also shown.

Figure 3. The voltage sensor model of Kv1.3-induced proliferation. Some of the elements of Kv1.3 channelosome that can participate in this process include integrin receptor, growth factor

(TK) receptors, caveolins, some scaffolding proteins and protein kinases such as MEK/ERK. Depolarization of V_M due to external and/or internal (the cell cycle) stimuli is sensed by Kv1.3 voltage sensor, and transduced into a conformational change of the channel intracellular domains that can promote phosphorylation and receptor or scaffolding protein associations, leading to the activation of pro-proliferative signaling pathways.

Figure 4. The channelosome model of Kv1.3-induced proliferation. The pro-proliferative role of Kv1.3 and the anti-proliferative role of Kv1.5 described in many preparations (see text) may be determining the assembly of Kv1.3 and Kv1.5 into homo- or heterotetrameric complexes, so that the relative proportion of both channels will control proliferation rate. Signaling of homotetrameric Kv1.3 channels will activate proliferation, while activation of Kv1.5 homotetramers will lead to an inhibition of proliferation. The pro-proliferative signals dependent on Kv1.3 activation will be occluded by the presence of Kv1.5 subunits in the heteromultimers, as Kv1.5 subunits will have a dominant-negative effect on Kv1.3-induced proliferation. Also, the biophysical differences between homo and heterotetrameric channels can play a role in cell proliferation.

Figure 5. Integrative flow of events between Kv1.3 channels and proliferation. Signals are initiated at the cell membrane by activation of Kv1.3 channels. Physically, the signal can be the K^+ flux, changes in V_M or volume changes. A number of processes act as receptors for these signals (including changes in intracellular ions, conformational changes activated by voltage or other physical protein interactions), allowing their transduction into biochemical changes, that are amplified through interaction and cross-talk with several proteins and signaling pathways leading to proliferation.

Figure 1A, Pérez-García MT et al

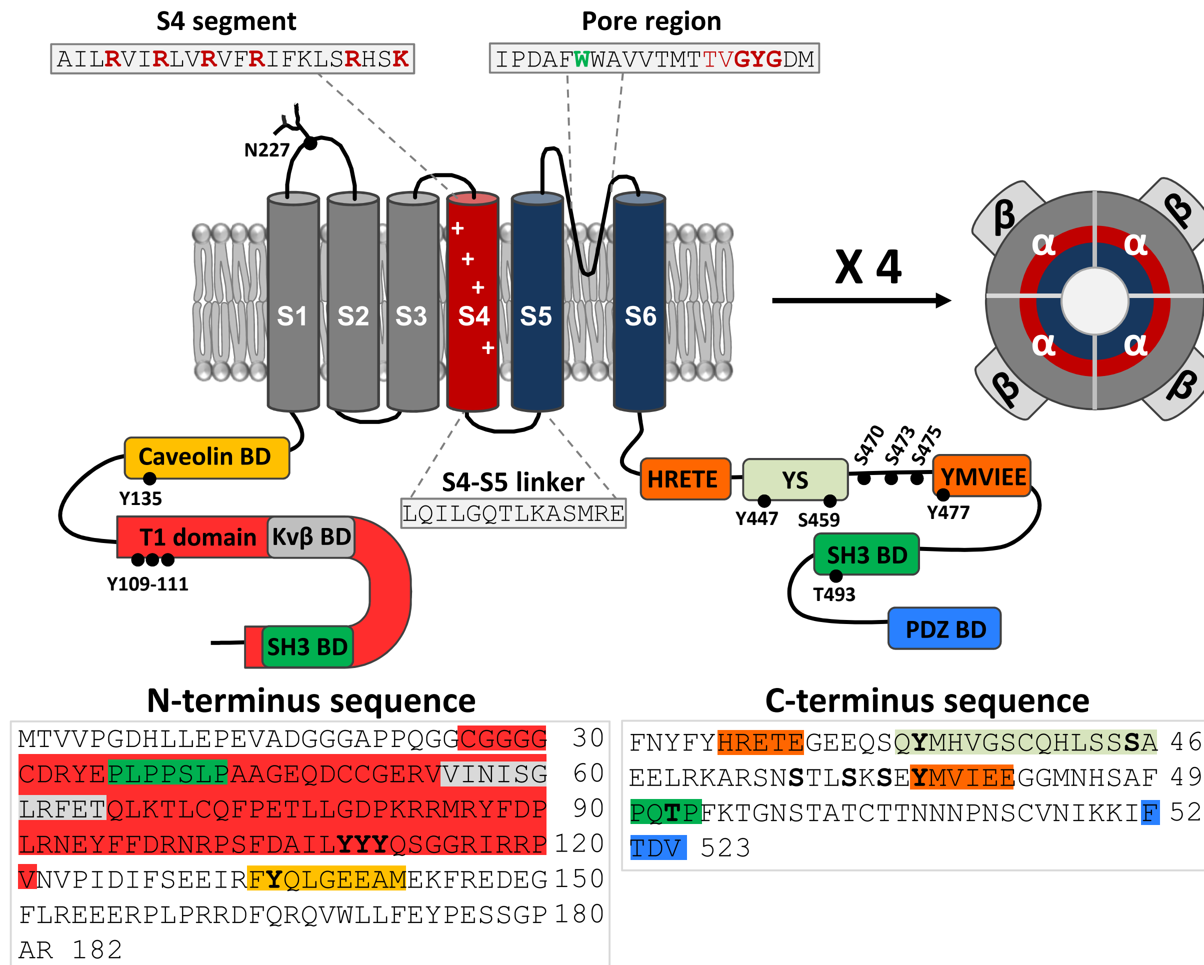
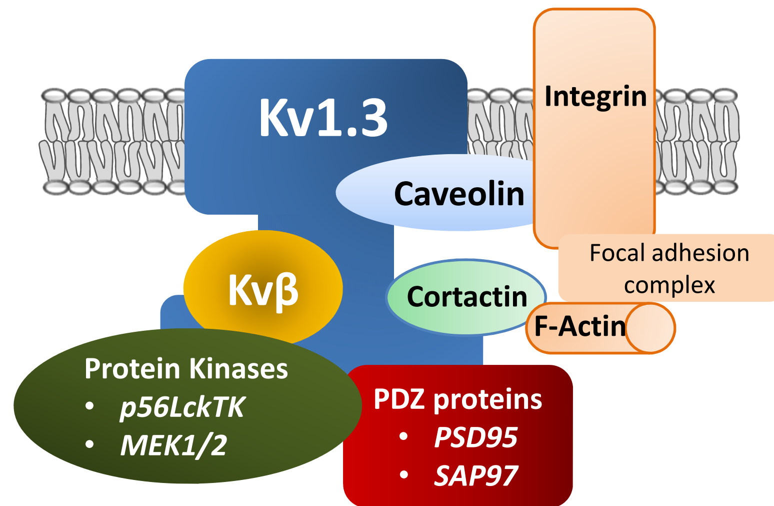


Figure 1B-C, Pérez-García MT et al



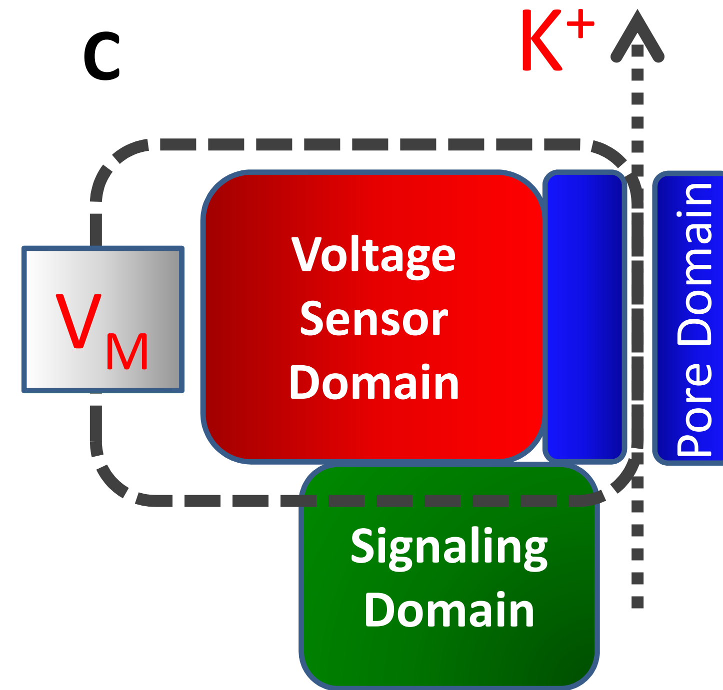
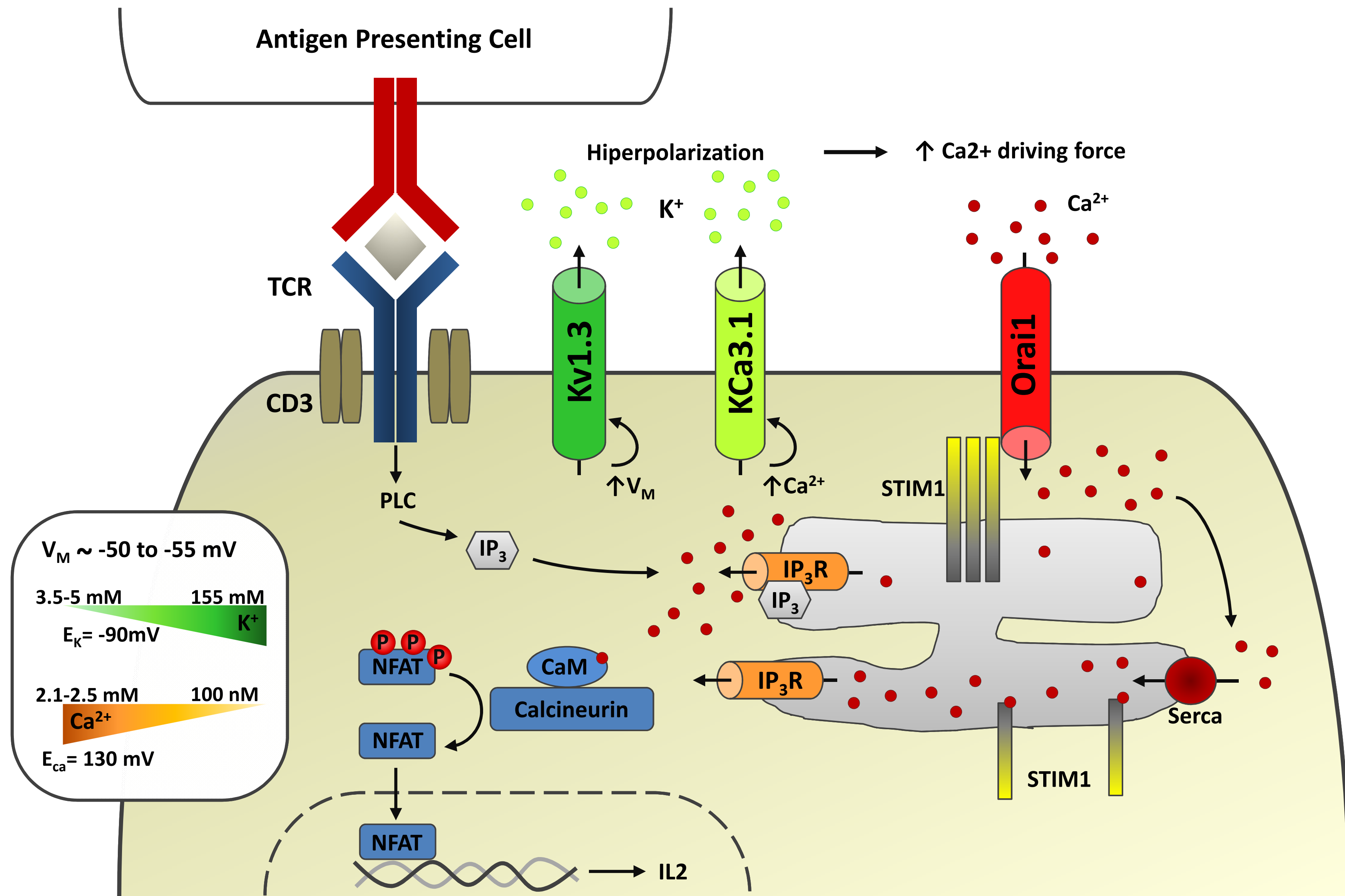


Figure 2, Pérez-García MT et al



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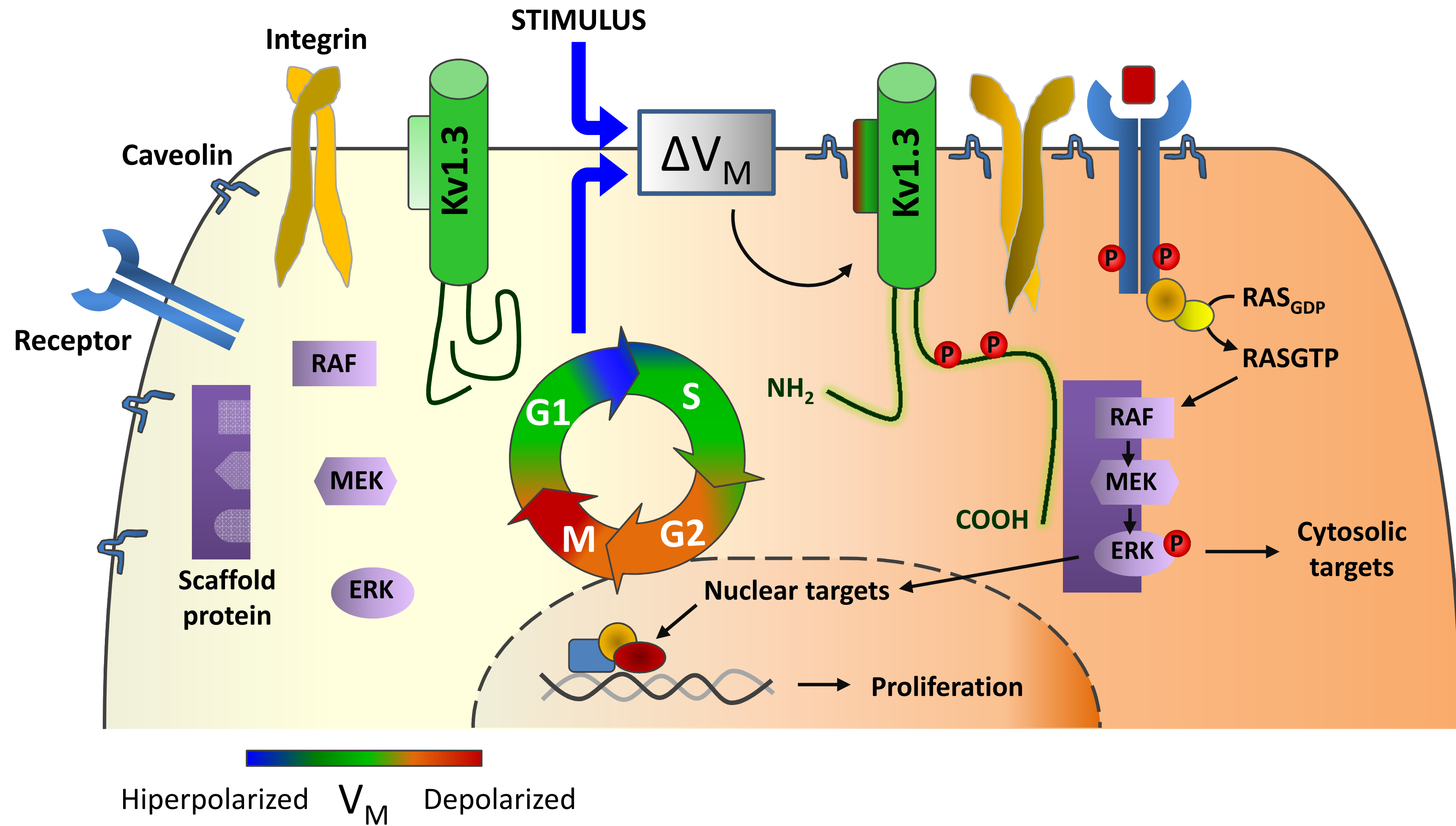

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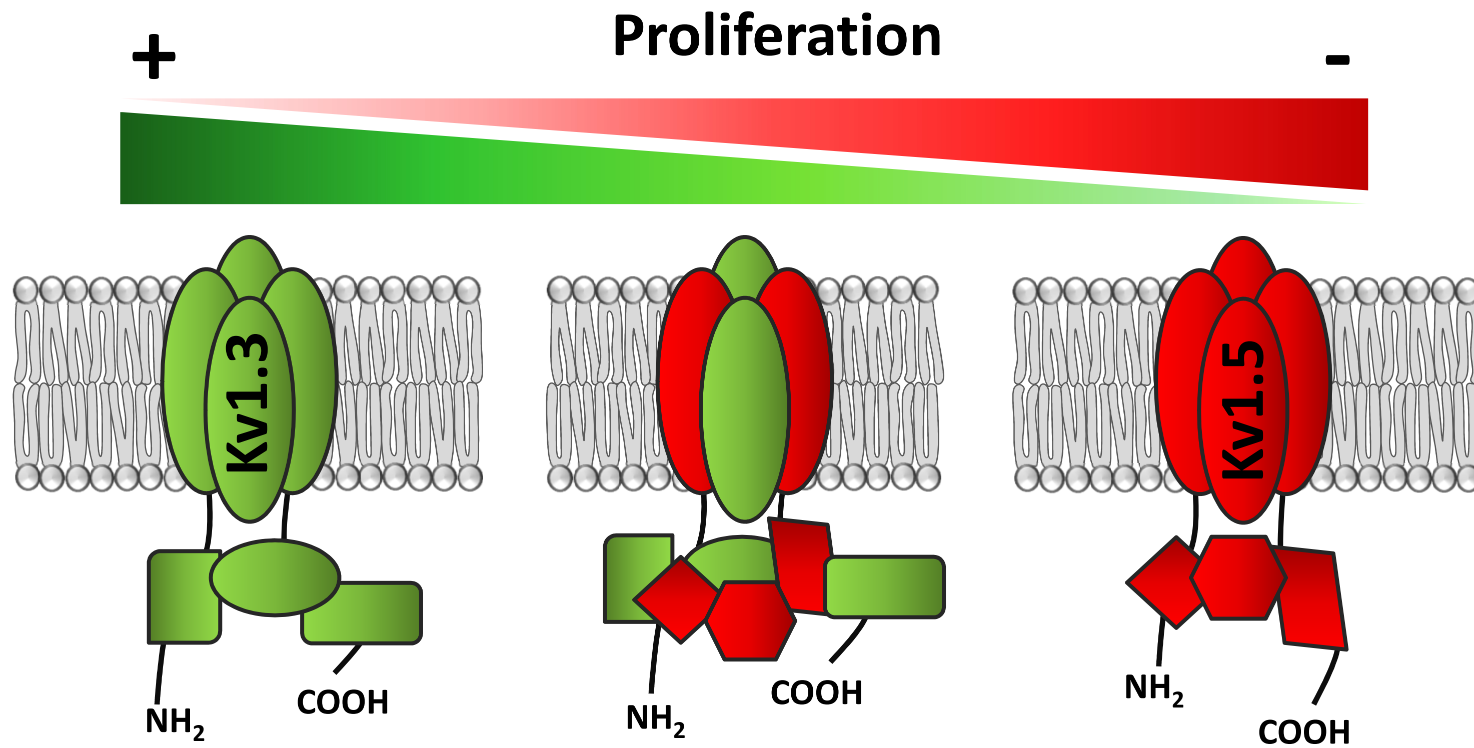

Figure 5, Pérez-García MT et al