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Molecular Substrates of Potassium Spatial Buffering in Glial Cells

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Abstract

It is generally accepted that the foremost mechanism for the buffering of K^+ from the extracellular space ($[K^+]_o$) in the brain is "K^+ spatial buffering." This is the process by which glial cells dissipate local K^+ gradients by transferring K^+ ions from areas of high to low $[K^+]_o$. These glial K^+ fluxes are mediated mainly by inwardly rectifying K^+ (Kir) channels. The K^+ spatial buffering hypothesis has been tested and confirmed in the retina, in which is has been termed as "K^+ siphoning". In Müller cells, the primary glial cells of the retina, Kir channels are distributed in a highly non-uniform manner, exhibiting high concentrations in membrane domains facing the vitreous humor (endfeet) and in proximity to the blood vessels (perivascular processes). Such non-uniform distribution of Kir channels facilitates directed K^+ fluxes in the retina from the synaptic plexiform layers to the vitreous humor and blood vessels. Recent molecular and electrophysiological studies in Müller cells have revealed a high degree of complexity in terms of Kir channel subunit composition, mechanisms of subcellular localization, and regulation. How such complexity fits into their proposed role in buffering $[K^+]_o$ in retina is the main topic of this article.

Index Entries: Potassium channel; potassium spatial buffering; glia; Müller cells; retina; dystrophin; syntrophin.

Glial Cells and Potassium Buffering

Numerous functions have been attributed to glial cells in the brain, including neurotransmitter clearance from synapses *(1)*, guidance during neuronal migration *(2)*, and more recently, active control of neuronal synaptic transmission *(3)*. Additionally, it has long been considered that glial cells within the central nervous system (CNS) help to maintain the optimal ionic environment required for communication within and between neurons. K^+ ions released as a result of neuronal activity can lead to substantial changes of extracellular K^+ concentration ($[K^+]_o$) given the limited volume of the extracellular space in the CNS *(4)* and relatively

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low baseline $[K^+]_o$ *(5).* Direct experimental evidence indicates that glial cells, more notably astrocytes, can effectively buffer or muffle changes in $[K^+]_o$ *(6).* The most widely accepted mechanism for such glial buffering of $[K^+]_o$ is the "K^+ spatial buffering" mechanism, first proposed by Orkand and colleagues in their landmark experiments in the optic nerve of amphibians *(7).* In their words, "if a glial cell becomes depolarized by K^+ that has accumulated in the clefts, the resulting current carries K^+ inward in the high $[K^+]_o$ region and out again, through electrically coupled glial cells in low $[K^+]_o$ regions." Thus, in this model, localized increases of $[K^+]_o$ in the CNS are dissipated through glial transfer of K^+ ions from areas of elevated $[K^+]_o$ to areas in which $[K^+]_o$ is low. Glial cells, which include astrocytes, oligodendrocytes, Schwann cells, and radial glia, are well-suited for K^+ spatial buffering, as they are endowed with unusual high membrane permeability to K^+ ions and they traverse long distances either by their elongated shape or by being electrically coupled to each other by gap junctions *(6).* Spatial buffering is expected to dissipate local accumulation of K^+ in a short time-scale (seconds), and it is believed to be more efficient than diffusion alone *(8,9).* Following the work by Orkand and colleagues, the spatial buffering mechanism has been demonstrated in various brain regions *(8,9)* and in great detail in the retina *(10).*

As expected, the high permeability to K^+ ions by glial-cell membranes is the result of the expression of a high density of K^+-selective channels with relatively high open probability at resting membrane potentials *(11).* These channels have the biophysical and pharmacological properties of the classical, inwardly rectifying K^+ (Kir) channels first described in frog skeletal muscle *(12)* and starfish or tunicate eggs *(13).* Kir channels are named for their attribute of allowing the passage of K^+ ions much more readily in the inward than the outward direction. These channels are expressed in a variety of tissue types in which they have an important role in regulating hormone release, cellular excitability, and potassium transport *(14–17).* An interesting feature of Kir channels is that they display a variable conductance, which correlates positively with the extracellular K^+ concentration *(18).* Therefore, increases in $[K^+]_o$ promote increases in Kir channel conductance through these channels, a feature believed to enhance the efficiency of the K^+ spatial buffering *(6).* Although other types of K^+ channels are found in glia such as calcium and voltage-dependent K^+ channels *(11),* those channels are mostly closed at the resting membrane potential of glial cells, which is usually very hyperpolarized (–60 to –90 mV). Thus, Kir channels provide the principal K^+ conductance in the major types of glia, and are believed to provide the pathways for K^+ spatial buffering in these cells.

Inwardly Rectifying K^+ Channels

The recent cloning of Kir channels has allowed investigators to address the question of their physiological role at the molecular level. More than twenty genes encode Kir channels *(17–19)* and their deduced primary amino acid sequences predict an overall topology consisting of two transmembrane domains, a reentrant loop (P-loop), and intracellular amino and carboxyl termini *(19).* Based on their degree of sequence identity, Kir channels are categorized into seven major subfamilies (Kir1 to Kir7) *(15,17,19).* Kir-channel subunits are highly conserved in their transmembrane domains, yet highly divergent in their amino and carboxyl termini. These channels lack the voltage sensor domain found in Kv channels; the voltage sensitivity of these channels arises from the voltage-dependent blockade of the inner pore by intracellular factors such as magnesium and polyamines *(16,17,20).*

An array of gating mechanisms are found among Kir channels. Kir3 (Kir3.1, Kir3.2, Kir3.3, and Kir3.4) and Kir6 (Kir6.1 and Kir6.2) channels are activated primarily by intracellular G-protein subunits *(21,22)* and decreases of ATP *(23),* respectively. Thus, these channels display low open probability at rest and open upon activation of G-protein-coupled receptors or from metabolic challenges to the cell. Since it is

unlikely that Kir3 and Kir6 channels play an important role in glial K^+ buffering under normal physiological conditions, they are not further considered here. The subunits in the Kir1 (Kir1.1) and Kir7 (Kir7.1) subfamilies are also unlikely to participate in K^+ spatial buffering in the brain, as their presence in glia is doubtful. They are expressed primarily in epithelial-cell types such as those in the kidney *(24–29),* choroid plexus *(30,31),* or retinal pigment epithelium (RPE) *(32,33).*

The channel subunits within Kir2 (Kir2.1, Kir2.2, Kir2.3, and Kir2.4), Kir4 (Kir4.1), and Kir5 (Kir5.1) subfamilies, on the other hand, are expressed in the brain and in glial-cell populations. Immunocytochemical and *in situ* hybridization studies show the expression of Kir4.1 subunits in both cultured and native astrocytes *(34–36),* and subpopulations of astrocytes in the cerebral cortex appear to display immunoreactivity to Kir4.1 subunits *(34,36).* In the olfactory bulb, Kir4.1 immunoreactivity is detected in about one-half of the glial fibrillary acidic protein (GFAP) positive astrocytes *(36).* Immunoelectron microscopic examination has revealed that Kir4.1 channels are enriched in the processes of astrocytes enveloping synapses and blood vessels *(36).* Kir2.2 channels are also immunolocalized to Bergman glial cells and astrocytes in the cerebellum *(37,38),* and Kir2.1 channel immunoreactivity was found in astrocytes and oligodendrocytes in the forebrain. Finally, in Müller cells, immunoreactivity toward Kir4.1 has revealed these channels both at light microscopy and electron microscopy levels *(39).* Recent studies have also demonstrated the expression of Kir2.1 and Kir5.1 subunits in this cell type *(40,41).* Thus, a number of immunocytochemical and *in situ* hybridization studies have demonstrated the astrocytic and Müller-cell expression of Kir2.1, Kir2.2, Kir4.1, and Kir5.1 subunits. A note of caution is that there is no complete agreement among these studies. For example, although one study shows the expression of Kir5.1 subunits in Müller cells *(41),* another fails to do so *(40).* Disparities in tissue fixation conditions or sensitivity and specificity of the antibodies used in these experiments may help to explain these discrepancies.

In the context of the K^+ spatial buffering function, we could ask about the expected physiological impact on a glial cell by expression of Kir channel subunits comprising Kir2.1, Kir4.1, and Kir5.1 channels. Most of the insights in this area come from studies in heterologous expression systems in which it has been possible to analyze properties of Kir channels derived from specified channel subunits without the confounding contribution of other endogenous currents. From these studies, it is clear that expression of Kir2.1, Kir4.1, and Kir5.1 subunits gives rise to currents with distinct functional properties. The Kir2.1 currents (as well as Kir2.2 and Kir2.3) show steep, inwardly rectifying current-voltage relationships with minimal outward currents at membrane potentials more positive than the K^+ equilibrium potential (E_K) *(42,43).* Kir4.1 channels, on the other hand, are weakly rectifying, allowing substantial outward currents at membrane potentials above E_K *(44).* The expression of Kir5.1 subunits in *Xenopus* oocytes or mammalian-cell lines does not result in the presence of functional channels, yet they are able to modify the properties of co-expressed Kir4.1 channels *(45,46).*

Kir channels are tetrameric proteins *(47),* and in heterologous expression systems, Kir subunits can form either homomeric or heteromeric channels. The evidence that glial cells can express more than one type of Kir-channel subunit also raises the question of whether these channels can co-assemble to form heteromeric channels in the native setting. Heteromeric assembly within a subfamily—such as channels from Kir2.1 and Kir2.3 subunits *(48)*—or across subfamilies such as Kir4.1/Kir5.1 heteromeric channels, display properties distinct from their homomeric channel counterparts *(46,49,50).* For example, Kir4.1/Kir5.1 channels display rectification properties, single-channel properties, and sensitivity to intracellular protons that are distinct from homomeric Kir4.1 channels *(45,49).* Immunoprecipitation assays using anti-Kir4.1 and Kir5.1 antibodies revealed potential heteromeric Kir4.1/Kir5.1 in Müller cells *(41);* however, to date, the extent to which the various Kir channel subunits func-

tion as homomers or heteromers in native astrocytes or Müller cells is unclear.

Kir-Channel Subtypes Expressed in Müller Cells

The now well-established functional heterogeneity of astrocytes *(51,52)* hampers a detailed molecular characterization of specific Kir channel subtypes in these cells. The Müller cell is functionally homogenous and readily accessible to electrophysiological measurements. Although astrocytes are found in the eye, they are restricted to a thin monolayer at the vitreal border at the inner surface of the retina, and are vastly outnumbered by Müller cells, which are the primary glial cell type in the retina *(53).*

The Müller cell is a polarized radial glial cell type that spans almost the entire width of the neural retina, with a club-like endfoot found at the vitreal border and apical processes that typically terminate at the level of the photoreceptor outer segments *(53,54).* Numerous lateral processes extend from the Müller cell in the somatic region, and are in close contact to the synaptic terminals in the inner and outer plexiform layers. In addition, in vascularized retinas (for example in the cat, monkeys, rats, or humans), Müller cells extend processes that ensheath blood vessels that pass through the retinal layers. The large size and easy accessibility of Müller cells in species such as salamanders or other amphibians has facilitated the study of these cells, and many of the finer details on the biophysical properties of K^+ conductances in Müller cells are derived from these studies. It was concluded that at the physiological membrane potential, the high K^+ conductance in Müller cells is dominated by Kir channels *(55–57).* As in other tissues, Müller cell Kir channels display inward rectification; their K^+ conductance is roughly proportional to the square root of $[K^+]_o$, and they are blocked by extracellular barium ions in a voltage-dependent manner. The presence of Kir channels with similar biophysical properties has been subsequently described for the guinea pig, rat, mouse, monkey, and human Müller cells *(58–62).* The high density of Kir channels in these cells is evident from the relatively low resting input resistance in the range of 50 to 100 MΩ.

Perhaps the most surprising finding from these studies was the apparent polarized and highly asymmetric distribution of Kir channels on Müller cell membranes. Patch clamp experiments *(55)* and the presentation of controlled focal elevations of $[K^+]_o$ at specific regions along the plasma membrane of these cells *(63,64)* revealed that Kir channels are not distributed in an uniform manner; rather, these channels are highly concentrated in "hot spots." In particular, Kir channels are most highly concentrated in the endfeet of Müller cells in retinas from all species examined, and also in the lateral processes of Müller cells in the species with vascularized retinas *(64).*

Such an asymmetric and highly polarized distribution of Kir conductance has led to the proposal by Newman of a specialized form of K^+ spatial buffering in the retina known as "K^+ siphoning" *(65).* In this model, K^+ ions released during synaptic activity in the inner and outer plexiform layers follow their electrochemical gradient and enter Müller cells, then leave the cells in the areas with highest K^+ conductance, such as the endfoot and/or perivascular processes. Because the endfoot lies adjacent to the vitreous humor, the K^+ released following neuronal activity is effectively shunted to a large sink compartment. This model was elegantly supported in experiments using frog eye cups in which light-evoked activity led to the expected K^+ increases in the inner plexiform layers as well as in the vitreous *(10).* Importantly, the increases in K^+ concentration in the vitreous were prevented upon retinal perfusion of barium, a potent blocker of Kir channel conductance.

More recently, various groups have examined the distribution of Kir channels in Müller cells using immunocytochemical and molecular biological techniques. Kurachi and colleagues were the first to demonstrate the expression of the weakly rectifying Kir4.1 channels in rat Müller cells *(66).* These channels are highly concentrated in endfeet and the processes enveloping the blood vessels (Fig. 1), a distribution that

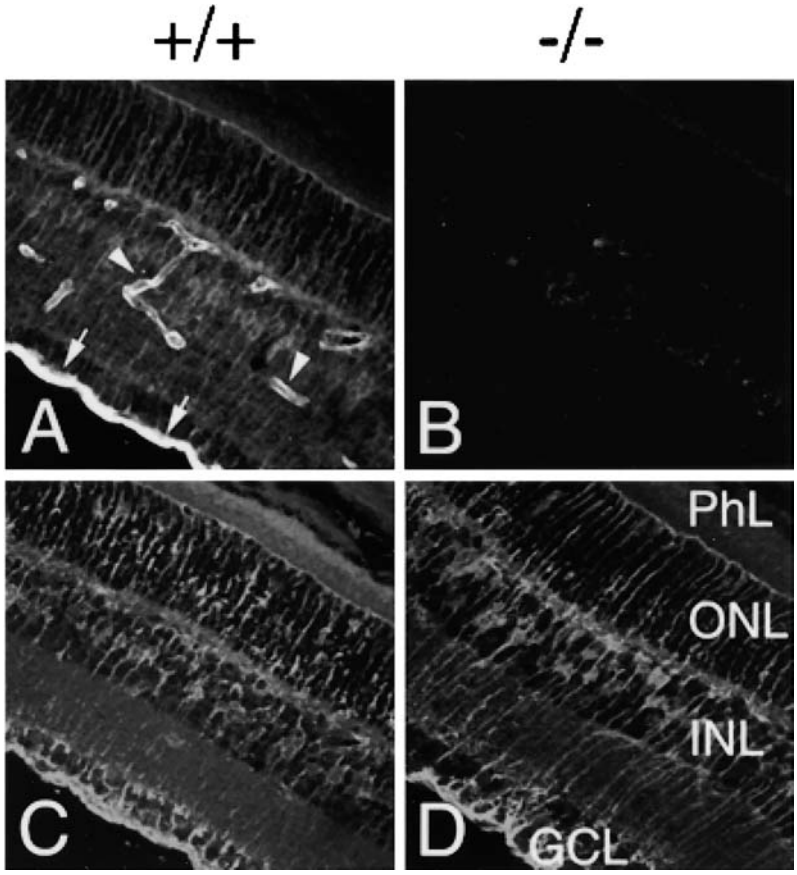

Fig. 1. Distribution of Kir4.1-channel immunoreactivity in retinal sections from wild-type and +/+ **(A,C)** and Kir4.1-null mice –/– **(B,D)**. Sections were double-stained with affinity-purified rabbit anti-rat Kir4.1 antibody, **(A,B)**, and monoclonal anti-GS antibody **(C,D)**. Observe the distribution of Kir4.1-channel immunoreactivity in the inner limiting membrane (arrows), as these channels are expressed in the endfeet of Müller cells and in membranes that surround blood vessels (arrowheads). GCL, ganglion-cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; PhL, photoreceptor layer. Adapted with permission from *(68).*

correlates well with the areas that demonstrated the highest K^+ conductances in electrophysiological studies. Several lines of experimental evidence argue that Kir4.1 subunits underlie the major Kir currents in Müller cells: i) patch-clamp recordings in rabbit Müller cells and in transfected HEK293 cells expressing the Kir4.1 channels show similar single channel conductance and open probability *(67),* ii) genetic ablation of Kir4.1 channels in mice showed that the membrane conductance of Müller cells was decreased by 10-fold, and are highly depolarized *(68),* and iii) the slow PIII wave in electroretinograms (ERGs), which has been associated with movements of K^+ ions upon light-evoked activity in retina is absent in Kir4.1 knockout animals *(68).* This effect was not caused by overall impairment of neuronal function, as shown by the fact that the a- and b-waves, which are associated to synaptic activity between rod and bipolar cells, were of comparable magnitude *(68).*

Although these studies revealed that Kir4.1 subunits are the principal Kir channel subunits in Müller cells, recent investigations have also provided evidence for expression of other sub-

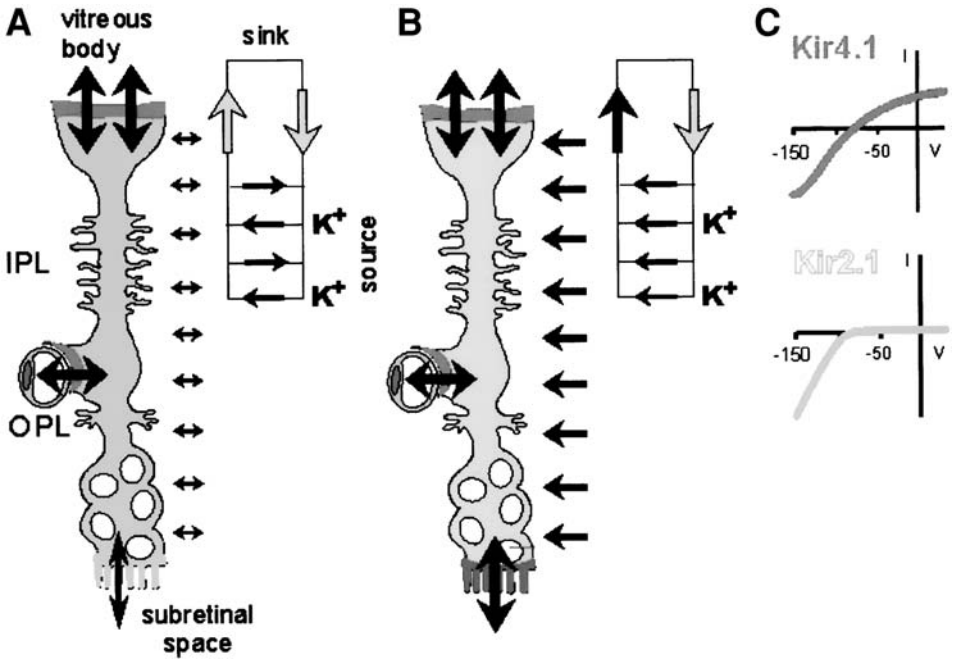

Fig. 2. Schematic drawing of the distribution of Kir-channel subtypes in Müller cells, and their contribution to retinal K^+ siphoning. **(A)** Classical view. Only weakly rectifying Kir channels are expressed, with high protein densities only in the endfeet and perivascular membranes. During retinal stimulation, the extracellular K^+ concentration is particularly elevated in the two synaptic layers, the inner (IPL) and outer plexiform layers (OPL). This causes a K^+ influx into Müller cells, and a simultaneous K^+ release into the vitreous humor, the blood vessels, and the subretinal space. The small simplified circuit (right side) exemplifies only a part of this mechanism, the siphoning from the source in the IPL into the vitreous body as the sink, and the backflow through the extracellular space. Since the Kir channels are rare in the membrane that faces the source (small arrows at the right side of the cell) and allow for both in- and outflow of K^+ (bidirectional arrows), the K^+ influx is rather small, and shunting outward currents may occur in the circuit. The K^+ efflux across the endfoot membrane is smaller than the local conductance would allow. **(B)** Novel model, based upon the presence and specific localization of two different Kir-channel subunits (Kir4.1, Kir 2.1). The weakly rectifying Kir channels (Kir4.1) are predominantly expressed in membranes through which K^+ ions flow out of Müller cells during light-evoked retinal activity. The strongly rectifying Kir2.1-channel subtype (unidirectional arrows) dominates the membranes that have contact to the perineuronal spaces (the K^+ sources), and which mediate the K^+ influx. The simplified circuit (right side) is now endowed with sufficient inward conductance at the source-faced areas, and lacks shunt currents; thus, the outward conductance can be used to full extend (black arrow). **(C)** Idealized current-voltage relationships of the two Kir channels. Although both Kir4.1 channels are open over a wide range of membrane potentials, and allow for inward and outward currents, Kir2.1 channels can open only at very negative membrane potentials, and are virtually perfect inward rectifiers. Adapted with permission from *(40).*

types within these cells. Immunocytochemical and PCR-based studies revealed that Müller cells also express the strongly rectifying Kir2.1 channels *(40).* Interestingly, the subcellular localization of Kir2.1 in Müller cells is quite distinct from that of Kir4.1. In the mouse, Kir2.1 channels are expressed along the plasma membrance of these cells in a seemingly uniform manner—e.g., without the hot spots seen in the distribution of Kir4.1 *(40).* We suggested that such differential distribution of Kir2.1 and Kir4.1 subunit channels enhances the efficiency of K^+ siphoning function in the retina (Fig. 2). In this model, weakly rectifying Kir channels

(Kir4.1) are expressed predominantly in membrane domains where K^+ ions leave the Müller cells for the extracellular sinks, whereas K^+ influxes from neuronal sources into the Müller cells are mediated mainly by the strongly rectifying Kir2.1 channels. The expression of Kir2.1 channels in membrane domains not facing the K^+ sinks may prevent the outward leak of K^+ from regions such as those with a high density of neurons in which efficient K^+ uptake into Müller cells is advantageous, yet efflux could be detrimental. Such spatial segregation of two Kir channel subunits with distinct biophysical properties provide a well designed example in which biophysical properties of individual ion channels and their localization are fine-tuned to their expected cellular function.

Additionally, one may imagine that the coordinated expression of two types of Kir channels could also provide an independent means of cellular regulation. Studies in heterologous expression systems have demonstrated that Kir4.1 channels are strongly modulated by changes in intracellular pH *(45,50).* The Kir4.1 channels are stimulated by intracellular alkalinization, with about 50% conductance increase resulting from changes of intracellular pH from pH 6–6.5. The Kir2.1 channels, on the other hand, are relatively insensitive to similar changes in intracellular pH *(45,50).* It has been demonstrated that light-evoked activity in the retina leads to neuronal induced alkalinization of the extracellular milieu *(53,54).* Müller cells counteract such alkalinization by acid efflux upon activation of Na/HCO_3 cotransporters, an activity that buffers the extracellular pH in the retina *(53,54).* We can speculate that acid efflux from Müller cells will also enhance the potassium siphoning activity in the retina by upregulation of Kir4.1 conductance. Such coupling of potassium and acid-base buffering activities in the retina by Müller cells may ensure the maintenance of an optimal ionic environment in the retina, even during periods of intense light activity. Interestingly, the acid efflux in Müller cells is greatest in their endfeet *(53,54),* where as discussed previously, Kir4.1 channels are mainly concentrated. Thus the biophysical properties and subcellular localization of Kir4.1 channels in Müller cells suggest these channels as effective targets for their modulation by intracellular pH.

More recently, another study has also suggested the expression of Kir5.1 channels in Müller cells *(41).* Immunoprecipitation assays show that at least a fraction of Kir4.1 subunits hetero-oligomerize with Kir5.1 subunits in the retina. Kir4.1/Kir5.1 hetero-oligomers are of particular interest, as they display sensitivity to intracellular pH at physiological range (pKa 6.8) (*45,50*). Thus, hetero-oligomerization of Kir4.1 and Kir5.1 subunits may provide the optimal range for intracellular pH sensitivity for Kir channels expressed in Müller cells. Thus far, the sensitivity of Kir conductance in Muller cells to changes in intracellular pH has not yet been verified experimentally.

In summary, there is compelling evidence to indicate a major role for Kir4.1 channels in the K^+ buffering function in the retina. Biochemical and immunocytochemical evidence also suggests that other Kir channels such as Kir2.1 and Kir5.1 may also provide additional and differentially modifiable pathways for K^+ entry into these cells.

Accessory Proteins to Kir Channels in Müller Cells: Localization and Function

The highly localized expression of Kir4.1 in Müller cells raises the question of how these channels are targeted and localized to their particular subcellular domains. The highly non-uniform distribution of this channel is paralled by the distribution of the water channel, aquaporin 4 (AQP4), which is also highly enriched in the endfeet and perivascular processes of Müller cells *(39).* Such tight co-localization of K^+ and water conductances may support the transfer of K^+ ions without causing large osmotic imbalances in the retina *(39).* Moreover, this co-localization also suggests a common molecular mechanism for their subcellular distribution and targeting. Although Kir4.1 and AQP4 channels are

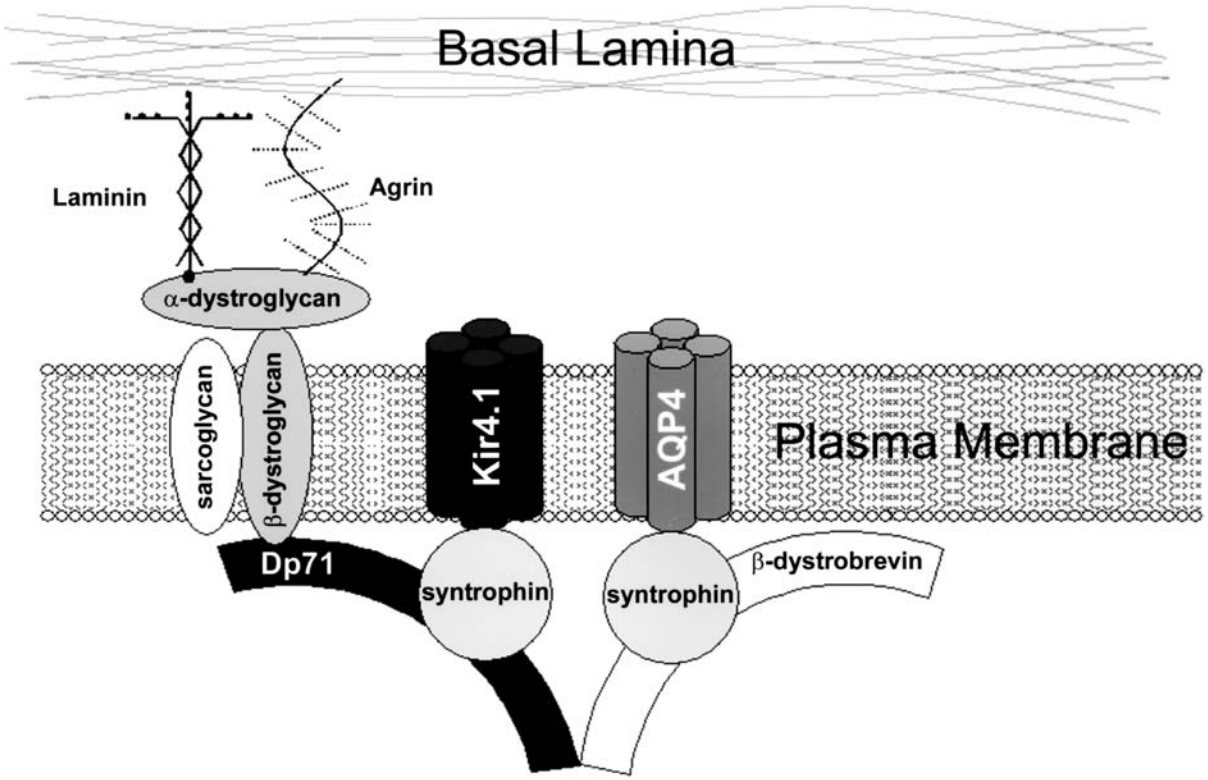

Fig. 3. Schematic representation of the Müller cell-specific dystrophin-glycoprotein complex (DGC). The complex is shown with the putative interaction between α-syntrophin and the inwardly rectifying potassium channel, Kir4.1, and the mercurial-insensitive water channel, AQP4.

highly divergent in their primary sequences, they share a key similarity in their C-termini, maintaining a -S-X-V-COOH motif. This sequence is identified for its ability to bind to PDZ domains, which are modular amino acid motifs implicated in many protein–protein interactions *(69,70).* Proteins possessing these domains are abundantly expressed in the nervous system and include postsynaptic density protein-95 (PSD-95), Chapsyn-110/PSD-93, SAP-102, and hDlg/SAP97.

Although specific PDZ domain-containing protein(s) in Müller cells have not yet been unequivocally identified, one possibility includes SAP97 *(71),* which was shown to be present in Müller cells and to increase currents evoked by Kir4.1 channels in heterologous systems *(71).* Another candidate is the PDZ domain-containing adapter protein, α-syntrophin.

In tissues in which α-syntrophin has been observed, it is localized to the cell membrane by its association with the multi-protein dystrophin glycoprotein complex (DGC) *(72,73).* The DGC spans the cell membrane, forming a molecular bridge from basal lamina proteins in the extracellular space, to an array of signaling molecules in the intracellular domain (Fig. 3). This complex is comprised of at least six different proteins, and has been described in astrocytes *(74)* and Müller cells *(75).* Perhaps the most notable protein in the DGC is dystrophin, which is present as one of many possible isoforms. Dystrophins are widely expressed in the CNS, and 20–40% of individuals with muta-

tions in the C-terminus exhibit non-progressive cognitive deficits *(76)* suggesting a significant role for these proteins in brain function. Moreover, those patients also display abnormalities when evoked field potential measurements from retina are observed, suggesting a possible role for dystrophins in retinal function as well *(77).*

Immunolocalization studies in the retina have revealed that the DGC components, α-dystroglycan *(78)* and the short dystrophin isoform, Dp71 *(79),* appear to be localized in a fashion that is very similar to that of Kir4.1 in Müller cells. Moreover, Dp71 appears to be required for the presence of normal retinal field potentials *(80).* The role for Dp71 in the localization of Kir4.1 was recently investigated by using the dystrophin null mutant mouse, mdx^{3Cv} *(81).* Immunohistochemistry experiments revealed that the polarized subcellular distribution of Kir4.1 is altered in Müller glial cells from mdx^{3Cv} mice, displaying a more homogeneous localization pattern (Fig. 4) *(82).* Despite this change, immunoblotting (Fig. 4) and whole-cell patch-clamp experiments have revealed that the channel is expressed at normal levels at the plasma membrane, and its electrophysiological properties are unchanged *(82).*

These results identify the DGC as an important means for the localization of Kir4.1 in Müller cells. The mechanism for targeting of Kir channels by the DGC to the membrane domains facing the vitreous or the blood vessels may occur through the binding of extracellular portions of the DGC to the basal lamina present in these regions (Fig. 3). The intracellular process involved in the localization of the channel assumes the existence of an intermediate protein with a PDZ domain. As mentioned previously, the best candidate for such an adaptor protein is α-syntrophin. First, α-syntrophin is expressed in Müller cells, and is putatively part of the Müller cell-specific DGC *(83)* (Fig. 3.) Secondly, α-syntrophin has been shown to interact with AQP4 in astrocytes in a PDZ-dependent manner, and to be required for the astrocytic membrane expression and localization of AQP4 *(72).* Therefore, α-syntrophin could underlie the co-localization of Kir4.1 and AQP4 seen in Müller cells. Furthermore, considering the ability for components of the DGC to bind signaling proteins such as Grb2, $PI(4,5)P_2$, and nNOS *(84),* it is conceivable that the DGC could act as a scaffold to keep signaling machinery and ion channels in close proximity as a functional unit, allowing for the intricate control of the state of cellular membrane properties.

Conclusion

In the past few years, the experimental evidence from a variety of studies has outlined a larger than anticipated degree of complexity in the structure and function of K^+ channels in glial cells. In Müller cells of the retina, K^+ conductances were determined to be asymmetrically distributed to their endfeet and perivascular processes. The "K^+ spatial buffering" mechanism was refined to "K^+ siphoning" to reflect the directional K^+ transport in the retina from the plexiform layers to the vitreous or blood vessels. Recent molecular studies indicate that Kir4.1 channel subunits are the molecular correlates for the highly asymmetric K^+ conductance found in Müller cells. Furthermore, Kir2.1—and possibly Kir5.1 subunits—are also expressed in Müller cells. The differential expression of Kir channel subunits in a single glial cell type was not anticipated from electrophysiological studies. K^+ buffering in the retina seems to be facilitated by concerted action of the strongly rectifying Kir2.1 channels to allow K^+ entry into the Müller cells and Kir4.1 channels to allow K^+ exit to the large sinks such as the vitreous humor. Furthermore, recent reports indicate that dystrophin and dystrophin-associated proteins promote the clustering and subcellular distribution of Kir4.1 channels in Müller cells. Future studies will undoubtedly continue to reveal deeper layers of glial complexity based on the possibility of multiple Kir subtypes being expressed in single cells, the prospect of their heteromerization to form channels with properties unique from their homomeric counterparts, and the ability

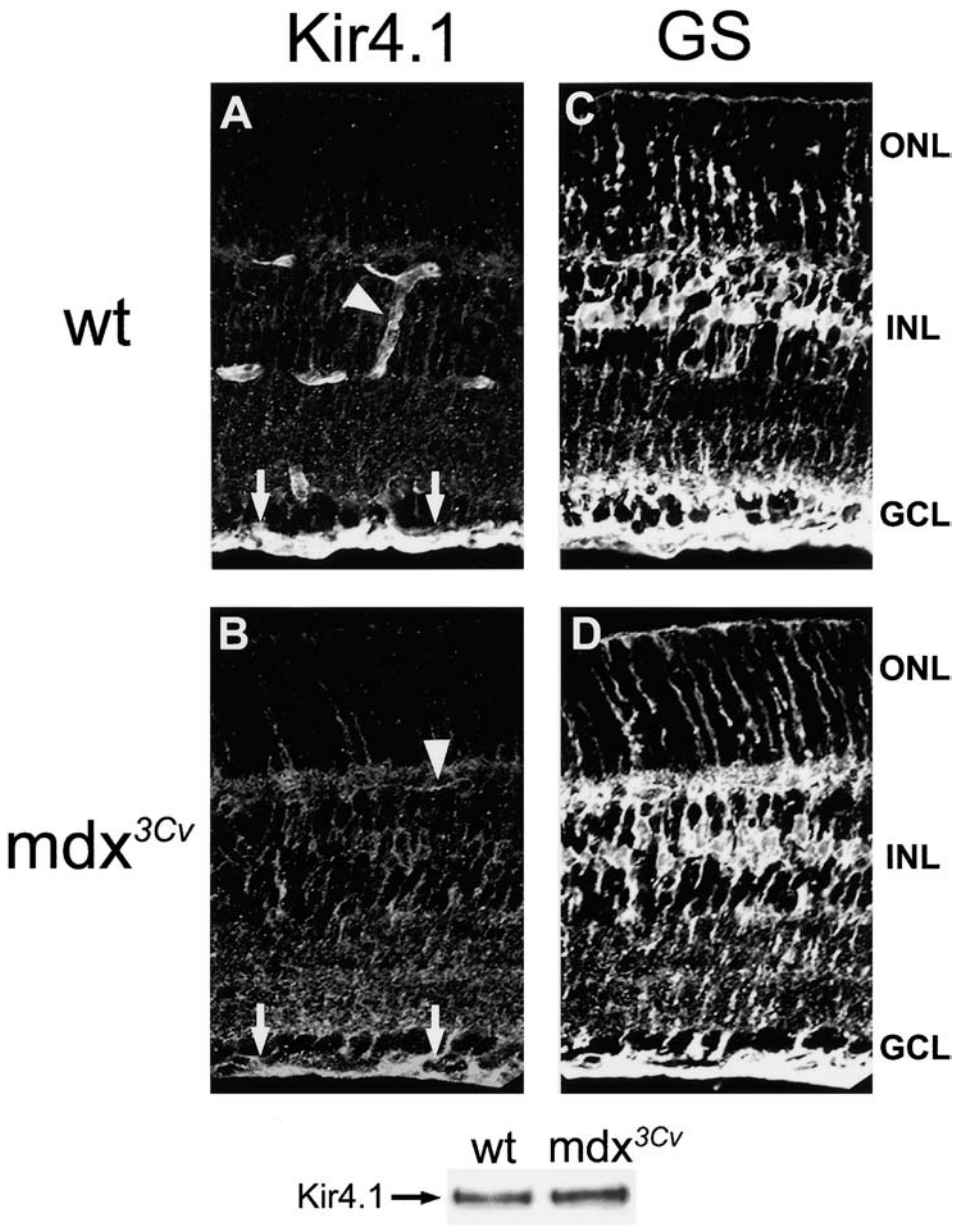

Fig. 4. The retinal distribution of Kir4.1 is mediated by the short dystrophin isoform Dp71. **(A)** In the wild-type mouse, Kir4.1 maintains a polarized distribution pattern, showing high expression at the endfeet (arrows) and in perivascular processes (arrowhead) of Müller cells. **(B)** immunolocalization of Kir4.1 in the dystrophin-null mutant (mdx^{3Cv}) revealed the elimination of the polarized distribution of the channel. In particular, note the relative reduction of Kir4.1 immunolocalization at the Müller-cell endfeet (arrows) and the perivascular processes (arrowhead) in the mutant mouse. **(C,D)** Immunolocalization of glutamine synthetase (GS), a marker for Müller cells, in the tissue shown in **A** and **B,** respectively. Bottom, Western blots revealed there to be no noticeable difference in the overall expression of Kir4.1 in the retina between the wild-type and mdx^{3Cv} mouse (ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion-cell layer). Adapted with permission from *(82).*

for their differential distribution by their ability to bind to different types of accessory proteins.

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