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The role of an inwardly rectifying K^+ channel (Kir4.1) in the inner ear and hearing loss

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Abstract

The *KCNJ10* gene which encodes an inwardly rectifying K^+ channel Kir4.1 subunit plays an essential role in the inner ear and hearing. Mutations or deficiency of *KCNJ10* can cause hearing loss with EAST or SeSAME syndromes. This review mainly focuses on the expression and function of Kir4.1 potassium channels in the inner ear and hearing. We first introduce general information about Kir potassium channels. Then, we review the expression and function of Kir4.1 channels in the inner ear, especially in endocochlear potential (EP) generation. Finally, we review *KCNJ10* mutation induced hearing loss and functional impairments. Kir4.1 is strongly expressed on the apical membrane of intermediate cells in the stria vascularis and in the satellite cells of cochlear ganglia. Functionally, Kir4.1 has critical roles in cochlear development and hearing through two distinct aspects of extracellular K^+ homeostasis: First, it participates in the generation and maintenance of EP and high K^+ concentration in the endolymph inside the scala media. Second, Kir4.1 is the major K^+ channel in satellite glial cells surrounding spiral ganglion neurons to sink K^+ ions expelled by the ganglion neurons during excitation. Kir4.1 deficiency leads to hearing loss with the absence of EP and spiral ganglion neuron degeneration. Deafness mutants show “loss-of-function” and reduced channel membrane-targeting and currents, which can be rescued upon by co-expression with wild-type Kir4.1. This review provides insights for further understanding Kir potassium channel function in the inner ear and the pathogenesis of deafness due to *KCNJ10* deficiency, and also provides insights for developing therapeutic strategies targeting this deafness.

Keywords

KCNJ10; endocochlear potential; spiral ganglion; deafness; EAST; SeSAME; cochlea

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1. Introduction

Inwardly rectifying potassium (Kir) channels exhibit an asymmetrical conductance at hyperpolarization (high conductance) compared to depolarization (low conductance) and demonstrate an inward rectification in the current-voltage relationship. They have various functions in setting the resting membrane potential, regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume regulation (Hille, 1992; Shieh et al., 2000). Kir channels also have a critical role in the inner ear for hearing. Kir4.1 is a predominant isoform in the inner ear. Mutations or knockout (KO) of Kir4.1 (*KCNJ10*) induce hearing loss. In this review, we mainly focus on reviewing the expression and function of Kir4.1 channels in the inner ear and mutation-induced functional impairments to provide insights for understanding the pathogenesis of deafness induced by *KCNJ10* deficiency.

2. Kir channel family and Kir4.1 channels

Inwardly rectifying K^+ currents were first identified in skeletal muscle (Katz, 1949). Later, the underlying Kir channels were identified and found to have widespread expression in a variety of cells, including cardiac myocytes (Kurachi, 1985; Nobles et al., 2005), neurons (Brown et al., 1990; Gahwiler and Brown, 1985; North et al., 1987; Takahashi, 1990), blood cells (McKinney and Gallin, 1988; Lewis et al., 1991), osteoclasts (Sims and Dixon, 1989), endothelial cells (Silver and DeCoursey, 1990), glial cells (Newman, 1984; Kofuji et al., 2002), and epithelial cells (Greger et al., 1990; Lorenz et al., 2002; Hebert et al., 2005). Based on molecular and electrophysiological features, the Kir channel family is divided into seven subfamilies (Kir1.0 – Kir7.0), which contain more than 20 members (Doupnik et al., 1995; Fakler and Ruppersberg, 1996; Isomoto et al., 1997; Nichols and Lopatin, 1997; Ruppersberg, 2000; Stanfield et al., 2002). On the other hand, based on their biophysical properties, Kir channels can also be divided into 4 sub-groups, namely: (1) Classical Kir channels (Kir2.x), (2) G protein-gated Kir channels (Kir3.x), (3) ATP-sensitive K^+ channels (Kir6.x), and (4) K^+-transport channels (Kir1.x, Kir4.x, Kir5.x, and Kir7.x).

Kir4.1 (*KCNJ10*) was initially identified from a brain cDNA library independently by several groups and has been assigned different names, such as BIR10 (Bond et al., 1994), KAB-2 (Takumi et al., 1995), BIRK-1 (Bredt et al., 1995), and Kir1.2 (Shuck et al., 1997). Kir4.1 has extensive expressions in glial cells of the brain (Higashi et al., 2001; Seifert et al., 2009; Harada et al., 2013), kidney (Garcia et al., 2007), and inner ear (Hibino et al., 1997, 1999; Ando and Takeuchi, 1999; Takeuchi et al., 2001).

3. Expression of Kir4.1 in the inner ear

Kir4.1 is a predominant isoform in the inner ear. In the inner ear, Kir4.1 mainly expresses in the cochlear lateral wall, the spiral ganglion area, and supporting cells in the organ of Corti (Hibino et al., 1997, 1999; Ando and Takeuchi, 1999; Takeuchi et al., 2001).

3.1 Kir4.1 expression in the stria vascularis in the cochlear lateral wall

Kir4.1 mRNA is the only detectable Kir channels in the stria vascularis (Hibino et al., 1997). Based on immunochemistry staining, they reported that Kir4.1 was located at the marginal cells. However, it was later found that Kir4.1 is expressed at the apical membrane of intermediate cells rather than the marginal cells (Ando and Takeuchi, 1999; Takeuchi et al., 2001). We also found that Kir4.1 labeling was only detectable in the intermediate cells and that no labeling was found in the marginal cells and the basal cells in dissociated cell preparations and in the cochlear cross-sections (Liu and Zhao, 2008; Wang et al., 2009). Currently, Kir4.1 is believed to be expressed only at the intermediate cells in the stria vascularis in the cochlear lateral wall (Fig. 1).

3.2 Kir4.1 expression in the cochlear ganglia

Using immunohistochemistry, Hibino et al. (1997, 1999) also found that Kir4.1 has prominent expression in the satellite glial cells of cochlear ganglia. The subcellular localization of Kir4.1 was further examined by immunoelectron microscopy. The Kir4.1 location was identified at the myelin sheaths of satellite glial cells wrapping the somata of the ganglion neurons (Hibino et al., 1999).

3.3 Kir4.1 expression in the organ of Corti

In the organ of Corti, weak-labeling for Kir4.1 was visible at Deiters' cells surrounding the outer hair cells (Rozengurt et al., 2003). This specific location implies that Kir4.1 may also play a role in K^+ absorption for K^+-recycling in the cochlea.

4. Kir4.1 function in the inner ear

Kir4.1 in the inner ear is mainly involved in the generation of positive endocochlear potential (EP) and high K^+ concentration in the endolymph. It also plays an important role in the maintenance of spiral ganglion neuron excitation

4.1 Kir4.1 function in the generation of endocochlear potential in the cochlear lateral wall

The EP is a driving force for hair cells to produce the auditory receptor current. The positive EP and high concentration of K^+ in the endolymph are generated by the cochlear lateral wall (Tasaki and Spyropoulos, 1959). Kir4.1 channels play an essential role in the generation of the EP. The cochlear lateral wall is composed of the stria vascularis (SV) and the spiral ligament (SPL). The stria vascularis comprises two epithelial layers: one layer of marginal cells and another composed of intermediate cells and basal cells (Fig. 1). Marginal cells are connected together through tight junctions forming a continuous layer facing the endolymph, whereas the layer of basal cells borders the spiral ligament that is filled with perilymph. Between the two layers there is an extracellular space termed the intrastrial space (IS) (Salt et al., 1987; Wangemann and Schacht, 1996; Wangemann 2006), which is surrounded by the apical membranes of intermediate cells and the basolateral membranes of marginal cells (Fig. 1B).

The IS is electrically isolated from the neighboring perilymph and endolymph and the fluid in the IS exhibits a low concentration of K^+ (1–2 mM) and a positive potential (+115–120

mV), which is 10–15 mV higher than the EP (Salt et al., 1987; Ikeda and Morizono, 1989). Early experiments indicated that this positive IS potential is generated by K^+ diffusion across the apical membranes of intermediate cells (Marcus et al., 1985; Salt et al., 1987; Wangemann and Schacht, 1996; Takeuchi et al., 1996; Takeuchi and Ando, 1998). Later, it was found that Kir4.1 channels located at the apical membrane of the intermediate cells are responsible for this K^+ permeability (Ando and Takeuchi, 1999; Takeuchi et al., 2001). In conjunction with a lower K^+ concentration (1–2 mM) in the IS and a normal high K^+ concentration (140–150 mM) in the cytosol of intermediate cells, Kir4.1 at the apical membrane of the intermediate cells can generate approximately 100–110 mV transmembrane potential. According to a widely-accepted "two-cell model" (Salt et al., 1987; Takeuchi et al., 2000; Marcus et al., 2002; Nin et al., 2008), the intermediate cells are electrically connected with the basal cells and neighboring fibrocytes in the SPL by gap junctions (Carlisle et al., 1990; Kikuchi et al., 1995; Lautermann et al., 1998; Forge et al., 2003; Liu and Zhao 2008; Kelly et al., 2011). Because the fibrocytes have high expression of Na^+, K^+-ATPase (Schulte and Adams, 1989; Schulte and Steel, 1994; ten Cate et al., 1994) and Na^+, K^+, $2Cl^-$-cotransporter (Crouch et al., 1997; Sakaguchi et al., 1998) that pump K^+ and Cl^- ions into the intracellular space leading to cells depolarizing, their intracellular potentials are approximately −5 mV (Melichar and Syka, 1987; Salt et al., 1987; Ikeda and Morizono, 1989; Nin et al., 2008). Consequently, the intracellular potential of the intermediate cells is driven to be at ~ −5 mV by gap junctional coupling. Because there is an approximately 100–110 mV transmembrane potential between the IS and the intracellular space of the intermediate cells generated by Kir4.1 potassium channels at the apical membrane of the intermediate cells, this eventually leads to the IS becoming positive and having a high positive potential (+105–115 mV) with respect to normal extracellular space (Fig. 2).

4.2 Function of Kir4.1 channels in the cochlear ganglia

Cochlear ganglion neurons belong to bipolar sensory neurons and are excited at their dendrites by neurotransmitters released from the hair cells. These neurons project to the cochlear nucleus and thus transfer auditory neural signaling from hair cells to the central auditory system. The somata of cochlear ganglion neurons are usually surrounded by more than two satellite glial cells (Rosenbluth, 1962; Ryan and Schwartz, 1983), as other types of sensory and autonomic ganglia. Electron microscopy shows that cochlear satellite glial cells wrap the somata of ganglion neurons with multiple layers of myelin sheaths, in which Kir4.1 is located (Hibino et al., 1999). This specific subcellular localization of Kir4.1 suggests that Kir4.1 channels in the satellite glial cells can sink K^+ ions, which are extruded from the ganglion neurons during excitation.

5. Kir4.1 expression in the inner ear development and functional maturation

5.1. Kir4.1 expression in the cochlear lateral wall and associated EP generation in the inner ear postnatal development

Both EP and the concentrations of K^+ ($[K^+]$) in cochlear endolymph gradually increase after birth. The EP starts to become positive after endolymphatic $[K^+]$ reaches the adult level around the postnatal day 8 (P8) in the rat (Hibino et al., 1997, 2004). The expression of

Kir4.1 in the SV starts after endolymphatic high [K^+] is established and increases to a plateau with a similar time course as the development of EP. In rats, Hibino et al. (1997) examined the developmental change of Kir4.1 immunoreactivity in SV at various postnatal days and compared it with the elevation of [K^+] and EP. At P1 and P5, no Kir4.1 immunoreactivity was detected. At P8, weak immunoreactivity was detected. The immunoreactivity appeared simultaneously in the SV in all turns of cochlea. The expression of Kir4.1 then increased rapidly during the following days. At P14, abundant staining of Kir4.1 protein was observed, similar to that of adult rats. The time course of Kir4.1 expression was closely correlated with that of the elevation of EP but not of endolymphatic [K^+], which further supports the idea that Kir4.1 has a critical role in EP generation.

In guinea pigs, Jin et al., (2006) investigated the developmental expression and localization of Kir4.1in the inner ear using semi-quantitative RT-PCR and immunohistochemistry. Kir4.1 expression was first observed in the strial intermediate cells at embryonic day 50, which suggests roles for Kir4.1 channels in inner ear development and onset of auditory function. However, the expression of Kir4.1 in the postnatal development of inner ears still remains largely undetermined and needs to be further investigated.

5.2. Postnatal development of Kir4.1 expression in the cochlear ganglia and auditory functional maturation

The expression of Kir4.1 in satellite cells also parallels maturation of auditory function during development. In rats, the function and structure of auditory systems, including the cochlea, are immature and nonfunctional just after birth. They develop gradually and become mature within 2 weeks, when the onset of hearing occurs in rats (Blatchley et al., 1987). Hibino et al., (1999) examined the developmental change of Kir4.1 immunoreactivity in cochlear satellite glial cells of rats at various postnatal days. At the first day after birth (P1) and P5, cochlear ganglion cells are small and poorly developed but the satellite glial cells are well-enveloped (Sobkowicz, 1992). No Kir4.1 labeling is visible in the satellite glial cells at P1 and P5. At P8, approximately when the myelin sheaths of the satellite glial cells begin to envelop the somata of ganglion neurons (Sobkowicz, 1992), weak immunoreactivity of Kir4.1 can be detected in some but not all satellite glial cells. The Kir4.1 immunoreactivity gradually increases in the following several days and reaches the adult level at P14. This developmental pattern of Kir4.1 expression appears the same in all turns of the cochlea. The onset of hearing in rats occurs at P14 (Blatchley et al., 1987), which is just after the complete maturation of Kir4.1 expression in the satellite glial cells and the strial intermediate cells in the stria vascularis. Similarly, in mice, the action potential of the cochlear nerve was first detected at P9, increased over several days, and reached a plateau at P14 (Mikaelian and Ruben, 1965; Sadanaga and Morimitsu, 1995), when hearing onset occurs. However, the relationship between auditory function and Kir4.1 expression in the mouse inner ear has not been completely established.

6. Hearing loss due to Kir4.1 (*KCNJ10*) deficiency

6.1 Deafness due to KCNJ10 mutations

Kir4.1 (*KCNJ10*) mutations can cause hearing loss. In humans, the *KCNJ10* gene is located on chromosome 1q22-23. *KCNJ10* mutations can produce autosomal recessive EAST syndrome, which entails epilepsy, ataxia, sensorineural deafness, and renal tubulopathy (Bockenhauer et al., 2009; Reichold et al., 2010; Freudenthal et al., 2011; Thompson et al., 2011), or SeSAME syndrome, characterized by seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (Scholl et al., 2009, 2012; Tang et al., 2010). Missense and nonsense mutations in *KCNJ10* have been identified in EAST/SeSAME syndromes. Bockenhauer et al. (2009) identified five children from two consanguineous families who had the clinical features of EAST syndrome. Sequencing of the complete coding region of *KCNJ10* revealed a homozygous missense mutation, c.194G → C (p.R65P), in the four affected patients in Family 1 and another homozygous missense mutation, c.229G →C (p.G77R), in Patient 2-1. Hearing impairment was found in Patient 1-1 at the age of 5 years and in Patient 2-1 at 1 year. The grade of hearing impairment in Patient 1-1 remained stable over the subsequent 8 years. Patients 1-3 and 1-4 also had sensorineural hearing impairment. The audiograms showed moderate hearing loss, which was more pronounced at higher frequencies.

Scholl et al. (2009) also screened the 4 kindreds with SeSAME syndrome and found homozygous missense mutations (p.C140R and p.T164I) in two consanguineous kindreds, compound heterozygous missense mutation (p.A167V/R297C) in one outbred kindred, and a compound missense/premature termination mutation (p.R65P/R199Stop) in one kindred.

Recently, three new homozygous mutations (p.R65C, p.F75L and p.F75C) and two new compound heterozygous mutations (p.V259fs259X and p.V91fs197X) in *KCNJ10* were also identified in patients with EAST syndrome (Freudenthal et al., 2011; Parrock et al., 2013). In addition, Parrock et al. (2013) found the homozygous mutation p.A167V, which had previously been reported to cause disease only in its compound heterozygous state (Williams et al., 2010), in an affected individual in one family; no other mutations were found in the entire coding sequence and adjacent splice sites.

6.2 The role of KCNJ10 mutations in enlarged vestibular aqueduct and Pendred syndrome

KCNJ10 mutations or deficiency may also be associated with enlarged vestibular aqueduct (EVA) and Pendred syndrome (PDS)/DNFB4 (Wangemann et al., 2004; Singh and Wangemann, 2008), which is an autosomal recessive syndromic deafness mainly caused by mutations of *SLC26A4*. The fact that large phenotypic variability exists among patients with biallelic *SLC26A4* mutations suggests that EVA/PDS is a complex disease and may involve other genetic or environmental factors influencing the clinical manifestations. Yang et al. (2009) screened 89 patients who had a clinical diagnosis of EVA/PS and were known carriers of only one *SLC26A4* coding sequence mutation. In two patients, they identified either p.P194H (c.581C) or p.R348C (c.1042C) missense mutations in *KCNJ10*. Neither change was found in 800 chromosomes from ethnically-matched normal-hearing controls.

However, a recent study (Landa et al., 2013) reported a lack of association between mutations of *KCNJ10* or *FOXI1* and *SLC26A4* mutations in EVA/PDS. They examined 68 patients with monoallelic mutations of *SLC26A4* who were tested for mutations in *KCNJ10* and *FOXI1*, and found no evidence for a significant association between *KCNJ10* or *FOXI1* mutations and *SLC26A4* (Landa et al., 2013). Song et al. (2014) also reported that there is no mutation identified in the *FOXI1*, *FOXI1-DBD*, and *KCNJ10* genes in 11 multiplex Korean families with EVA and hearing loss that carry biallelic mutations of the *SLC26A4* gene. There were also no *FOXI1* or *KCNJ10* mutations detected in 32 EVA Chinese patients who carried pathogenic *SLC26A4* mutations (Chen et al., 2012). In addition, Jonard et al., (2010) reported that there were no mutations in *FOXI1* and *KCNJ10* genes in 25 patients presenting unilateral hearing impairment and ipsilateral EVA. So, the possible digenic inheritance of PDS due to single allele mutations in both *KCNJ10* and *SLC26A4* has not been firmly established. The role of *KCNJ10* mutations in EVA needs further clarification in future studies.

7. Functional analyses of *KCNJ10* deafness mutations

So far, deafness alleles of homozygous p.T57I, p.R65P, p.R65C, p.F75C, p.F75L, p.G77R, p.C140R, p.T164I, p.A167V, p.R175Q, and p.R297C, compound heterozygous p.R65P/R199Stop and p.A167V/R297C, and frame-shift mutations of p.V91fs197X and p.V259fs259X have been identified (Bockenhauer et al., 2009; Scholl et al., 2009, 2012; Freudenthal et al., 2011; Parrock et al., 2013). For functional assay, these mutants and wildtype (WT) *KCNJ10* constructs were cloned and expressed in Xenopus oocytes (Williams et al., 2010, Freudenthal et al., 2011; Parrock et al., 2013) and mammalian cell lines (HEK293 and CHO cells: Reichold et al., 2010; HEK293 cells: Tang et al., 2010; Williams et al., 2010; Scholl et al., 2012; COSm6 cells: Sala-Rabanal et al., 2010). All of the mutations compromised channel function, but the underlying mechanisms are different.

7.1 Impairment in Kir4.1 membrane targeting

Kir4.1 is a membrane protein. Cell-surface membrane targeting assays indicate that all mutants with the exception of the nonsense mutant p.R199X, exhibit plasma membrane expression, however reduced (Tang et al., 2010; Sala-Rabanal et al., 2010; Williams et al., 2010, Scholl et al., 2012). The p.A167V mutant also can decrease surface expression in p.A167V/R297C (Sala-Rabanal et al., 2010).

7.2 Impairment in Kir4.1 channel function

All of the disease-associated mutants (p.T57I, p.R65P, p.R65P/R199X, p.G77R, p.C140R, p.T164I, p.A167V, p.A167V/R297C, p.R175Q, and p.R199X) show loss-of-function mutations and reduced K^+ current to 0–23% of the WT level (Reichold et al., 2010; Tang et al., 2010; Sala-Rabanal et al., 2010; Williams et al., 2010, Scholl et al., 2012; Parrock et al., 2013). A majority of mutations (p.R297C, p.C140R, p.R199X, and p.T164I) resulted in complete loss of Kir4.1 channel function (Tang et al., 2010). Single-channel analysis revealed that p.R65P, p.G77R, and p.R175Q mutations reduced channel activity by decreasing channel mean open time (Reichold et al., 2010). It has also been found that p.R65P, p.T164I, p.R175Q, and p.R297C mutants can cause a remarkable shift of pH

sensitivity to the alkaline range (Reichold et al., 2010; Sala-Rabanal et al., 2010). Reducing pH from approximately 7.4 to 6.8 significantly decreased currents of all tested-mutants (p.R65P, p.R65P/R199X, p.G77R, p.C140R, p.T164I, and p.A167V/R297C) except p.R199X, but did not affect WT channels (Williams et al., 2010). This leads to the hypothesis that perturbed pH gating may underlie the loss of channel function in the disease-associated Kir4.1 mutants and may have important physiologic consequences (Williams et al., 2010).

7.3 Impairment in function of Kir4.1/Kir5.1 heteromers

A Kir4.1 channel is composed of four isoforms. Besides homomeric channel configuration, Kir4.1 can assemble with Kir5.1 (*KCNJ16*) to form heteromeric channel configurations, which are required for Na/K-ATPase pump activity. The co-localization of Kir4.1 and Kir5.1 was found in the basolateral membrane of the cortical thick ascending limb in the kidney (Lourdel et al., 2002; Lachheb et al., 2008) and Muller cells in the retina (Kofuji et al., 2000; Ishii et al., 2003) but not in the cochlea (Hibino et al., 2004). When the aforementioned mutants were co-expressed with Kir5.1, currents remained reduced, similar to that as mutants were expressed alone (Reichold et al., 2010; Tang et al., 2010; Williams et al., 2010; Parrock et al., 2013). This suggests that Kir4.1 isoforms in Kir4.1/Kir5.1 (*KCNJ10/KCNJ16*) heteromers may play a dominant role in channel function.

8. Kir4.1KO mouse model and associated pathology in the inner ear

Kir4.1 KO mice were established by genetic deletion (Kofuji et al., 2000). The coding exon (amino acids 33–266) in the *KCNJ10* sequence was deleted and replaced with a neomycin resistance gene. The deleted fragment contains the putative transmembrane domain (TM) and part of the C-terminus. Most of the homozygous mice could survive up to 3 weeks after birth (Kofuji et al., 2000).

The absence of *KCNJ10* gene expression in the inner ear in homozygous KO mice was confirmed by immunolabeling (Rozengurt et al., 2003). Kir4.1 KO mice have profound deafness with the absence of the EP and loss of endolymphatic K^+ (Marcus et al., 2002; Rozengurt et al., 2003). In Kir4.1 KO mice, Reissner's membrane is collapsed, the tectorial membrane is swollen, and inner and outer hair cells degenerate over the first postnatal weeks (Rozengurt et al., 2003). These pathological changes in the Kir4.1 KO mouse agree with the spatial and temporal distribution of Kir4.1 in the cochlea.

In the Kir4.1 KO mice, spiral ganglion neurons as well as their central processes also show quick degeneration after birth (Rozengurt et al., 2003). Kir4.1 is located at the myelin sheathes of satellite glial cells but not at the spiral ganglion neurons (Hibino et al., 1997, 1999, 2004). The satellite glial cells wrap the somata of the spiral ganglion neurons and share many similarities with brain glial cells. Both of them have high expression of Kir4.1 (Hibino et al., 1997, 1999; Higashi et al., 2001; Seifert et al., 2009; Harada et al., 2013). It has been reported that Kir4.1 channels are crucial for oligodendrocyte maturation and oligodendrocyte-dependent myelination of axons in the spinal cord (Neusch et al., 2001). Kir4.1 knockout results in oligodendrocyte depolarization, dysmyelination or demyelination, and a failure to form compact myelin sheaths. This eventually results in axonal degeneration

of neurons in the spiral cord (Neusch et al., 2001). Kir4.1 may also play the same role in the maturation and myelination of the spiral ganglion neurons in the inner ear. Deficiency of Kir4.1 may cause satellite glial cell depolarization and spiral ganglion neuron dysmyelination as well, leading to eventual spiral ganglion neuron degeneration (Rozengurt et al., 2003). However, such hypothesis currently lacks evidence. Further studies are required.

9. Functional rescue of *KCNJ10* deficiency

The channel function of Kir4.1 mutations can be rescued upon by co-expression with WT *KCNJ10* (Tang et al., 2010; Sala-Rabanal et al., 2010). Co-expression of mutants with WT *KCNJ10* restored channel function for p.R65P, p.T164I, p.A167V, and p.R297C mutations. For p.G77R and p.C140R mutants, the channel function was not fully restored by co-expression of WT *KCNJ10*; a partial dominant negative effect was detected (Williams et al., 2010). The restoration of channel function was also observed in p.R65C, p.F75L, and p.V259fs259X mutations with co-expression of WT *KCNJ10* (Freudenthal et al., 2011). It has been reported that p.R199Stop reduced surface expression, but channel activity could be restored by co-expression with WT *KCNJ10* (Sala-Rabanal et al. 2010). However, so far, no functional rescue data are available in intact animals.

10. Implication of mutations on Kir4.1channel structure-function

The membrane topology of *KCNJ10* consists of two transmembrane helices and intracellular N- and C-termini (Fig. 3). Disease alleles are located at the first and second transmembrane helices and N- and C-termini. Functional analyses (Sala-Rabanal et al. 2010; Reichold et al., 2010) show that p.R65P, p.T164I, and p.R297C, located at N-terminus near the first transmembrane domain (TM1), the cytoplamic site of the second transmembrane domain (TM2), and C-terminus, respectively, caused an alkaline shift in pH sensitivity, indicating that these positions are crucial for pH sensing and pore gating. Mutant p.G77R locates at the middle of the TM1 and introduces a positive charge within the bilayer, which may be able to affect channel structure or gating. Mutant p.C140R that locates at the extracellular side of the TM2 breaks the Cys(108)–Cys(140) disulfide bond, which is essential for protein folding and function. Mutant p.R297C at the C-terminus disrupts the intersubunit salt bridge Glu(288)-Arg(297). Mutant p.R199Stop at C-terminus also dramatically reduced surface expression. However, the channel activity could be restored by co-expression with WT Kir4.1 (Sala-Rabanal et al. 2010).

11. Conclusions and prospects

In summary, Kir4.1 is vital for inner ear physiology and hearing. Kir4.1 is strongly expressed in the intermediate cells of stria vascularis and the satellite glial cells of cochlear ganglia and has two major roles in the inner ear. First, Kir4.1 in the stria vascularis participates in generating and maintaining positive EP in the endolymph. Second, Kir4.1 is the major K^+ channel in satellite glial cells surrounding spiral ganglion somas and axons and possibly in epithelial cells surrounding hair cells and serves to sink expelled K^+ during excitation maintaining these cells excitable. Deficiency of Kir4.1 functions can cause

hearing loss and is associated with EAST syndrome or SeSAME syndrome. Functional assays reveal that deafness mutants show “loss-of-function” and reduced channel current. The deficient channel function can be rescued by co-expression with wildtype *KCNJ10*. However, the mechanisms underlying these functions and deafness still remain largely unclear. For example, a recent study reveals that aging mice have lower levels of *KCNJ10* expression in the cochlear lateral wall but retained the normal level of EP with a reduction of expression of Na-K-ATPase and NKCC1 potassium transporters (Yang et al., 2013). This result suggests that Kir4.1 function requires coordination of other potassium transporter performance and its expression may be dependent on K^+ influx and efflux.

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References

Ando M, Takeuchi S. Immunological identification of an inward rectifier K^+ channel (Kir4.1) in the intermediate cell (melanocyte) of the cochlear stria vascularis of gerbils and rats. Cell Tissue Res. 1999; 298:179–183. [PubMed: 10555552]

Blatchley BJ, Cooper WA, Coleman JR. Development of auditory brainstem response to tone pip stimuli in the rat. Dev Brain Res. 1987; 32:75–84.

Bockenhauer D, Feather S, Stanescu HC, Bandulik S, Zdebik AA, Reichold M, Tobin J, Lieberer E, Sterner C, Landoure G, Arora R, Sirimanna T, Thompson D, Cross JH, van't Hoff W, Al Masri O, Tullus K, Yeung S, Anikster Y, Klootwijk E, Hubank M, Dillon MJ, Heitzmann D, Arcos-Burgos M, Knepper MA, Dobbie A, Gahl WA, Warth R, Sheridan EK, et al. Epilepsy, Ataxia, Sensorineural Deafness, Tubulopathy, and *KCNJ10* Mutations. N Engl J Med. 2009; 360:1960–1970. [PubMed: 19420365]

Bond CT, Pessia M, Xia XM, Lagrutta A, Kavanaugh MP, Adelman JP. Cloning and expression of a family of inward rectifier potassium channels. Receptors Channels. 1994; 2:183–191. [PubMed: 7874445]

Bredt DS, Wang TL, Cohen NA, Guggino WB, Snyder SH. Cloning and expression of two brain-specific inwardly rectifying potassium channels. Proc Natl Acad Sci USA. 1995; 92:6753–6757. [PubMed: 7624316]

Brown DA, Gahwiler BH, Griffith WH, Halliwell JV. Membrane currents in hippocampal neurons. Prog Brain Res. 1990; 83:141–160. [PubMed: 2203096]

Carlisle L, Steel K, Forge A. Endocochlear potential generation is associated with intercellular communication in the stria vascularis: structural analysis in the viable dominant spotting mouse mutant. Cell Tissue Res. 1990; 262:329–337. [PubMed: 2076537]

Chen K, Wang X, Sun L, Jiang H. Screening of SLC26A4, FOXI1, KCNJ10, and GJB2 in bilateral deafness patients with inner ear malformation. Otolaryngol Head Neck Surg. 2012; 146:972–978. [PubMed: 22412181]

Crouch JJ, Sakaguchi N, Lytle C, Schulte BA. Immunohistochemical localization of the Na-K-Cl co-transporter (NKCC1) in the gerbil inner ear. J Histochem Cytochem. 1997; 45:773–778. [PubMed: 9199662]

Doupnik CA, Davidson N, Lester HA. The inward rectifier potassium channel family. Curr Opin Neurobiol. 1995; 5:268–277. [PubMed: 7580148]

Fakler B, Ruppersberg JP. Functional and molecular diversity classifies the family of inward-rectifier K^+ channels. Cell Physiol Biochem. 1996; 6:195–209.

Forge A, Becker D, Casalotti S, Edwards J, Marziano N, Nevill G. Gap junctions in the inner ear: comparison of distribution patterns in different vertebrates and assessement of connexin composition in mammals. J Comp Neurol. 2003; 467:207–231. [PubMed: 14595769]

Freudenthal B, Kulaveerasingam D, Lingappa L, Shah MA, Brueton L, Wassmer E, Ognjanovic M, Dorison N, Reichold M, Bockenhauer D, Kleta R, Zdebik AA. KCNJ10 mutations disrupt function in patients with EAST syndrome. Nephron Physiol. 2011; 119:40–48.

Gahwiler BH, Brown DA. $GABA_B$-receptor-activated K^+ current in voltage clamped CA3 pyramidal cells in hippocampal cultures. Proc Natl Acad Sci USA. 1985; 82:1558–1562. [PubMed: 2983351]

Garcia MA, Meca R, Leite D, Boim MA. Effect of renal ischemia/reperfusion on gene expression of a pH-sensitive K^+ channel. Nephron Physiol. 2007; 106:1–7.

Greger R, Bleich M, Schlatter E. Ion channels in the thick ascending limb of Henle's loop. Ren Physiol Biochem. 1990; 13:37–50. [PubMed: 1689860]

Harada Y, Nagao Y, Shimizu S, Serikawa T, Terada R, Fujimoto M, Okuda A, Mukai T, Sasa M, Kurachi Y, Ohno Y. Expressional analysis of inwardly rectifying Kir4.1 channels in Noda epileptic rat (NER). Brain Res. 2013; 1517:141–149. [PubMed: 23603404]

Hebert SC, Desir G, Giebisch G, Wang W. Molecular diversity and regulation of renal potassium channels. Physiol Rev. 2005; 85:319–371. [PubMed: 15618483]

Hibino H, Horio Y, Inanobe A, Doi K, Ito M, Yamada M, Gotow T, Uchiyama Y, Kawamura M, Kubo T, Kurachi Y. An ATP-dependent inwardly rectifying potassium channel, KAB-2 (Kir4. 1), in cochlear stria vascularis of inner ear: its specific subcellular localization and correlation with the formation of endocochlear potential. J Neurosci. 1997; 17:4711–4721. [PubMed: 9169531]

Hibino H, Horio Y, Fujita A, Inanobe A, Doi K, Gotow T, Uchiyama Y, Kubo T, Kurachi Y. Expression of an inwardly rectifying K^+ channel, Kir4.1, in satellite cells of rat cochlear ganglia. Am J Physiol. 1999; 277:C638–C644. [PubMed: 10516093]

Hibino H, Higashi-Shingai K, Fujita A, Iwai K, Ishii M, Kurachi Y. Expression of an inwardly rectifying K^+ channel, Kir5.1, in specific types of fibrocytes in the cochlear lateral wall suggests its functional importance in the establishment of endocochlear potential. Eur J Neurosci. 2004; 19:76–84. [PubMed: 14750965]

Higashi K, Fujita A, Inanobe A, Tanemoto M, Doi K, Kubo T, Kurachi Y. An inwardly rectifying K^+ channel, Kir4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. Am J Physiol Cell Physiol. 2001; 2813:C922–C931. [PubMed: 11502569]

Hille, B. Ionic channels in excitable membranes. 2nd ed.. Sutherland: Sinauer; 1992. p. 127-130.

Ikeda K, Morizono T. Electrochemical profiles for monovalent ions in the stria vascularis: Cellular model of ion transport mechanisms. Hear Res. 1989; 39:279–286. [PubMed: 2753832]

Ishii M, Fujita A, Iwai K, Kusaka S, Higashi K, Inanobe A, Hibino H, Kurachi Y. Differential expression and distribution of Kir5.1 and Kir4.1 inwardly rectifying K^+ channels in retina. Am J Physiol Cell Physiol. 2003; 285:C260–C267. [PubMed: 12686518]

Isomoto S, Kondo C, Kurachi Y. Inwardly rectifying potassium channels: their molecular heterogeneity and function. Jpn J Physiol. 1997; 47:11–39. [PubMed: 9159640]

Jin Z, Wei D, Jarlebark L. Developmental expression and localization of *KCNJ10* K^+ channels in the guinea pig inner ear. Neuroreport. 2006; 17:475–479. [PubMed: 16543810]

Jonard L, Niasme-Grare M, Bonnet C, Feldmann D, Rouillon I, Loundon N, Calais C, Catros H, David A, Dollfus H, Drouin-Garraud V, Duriez F, Eliot MM, Fellmann F, Francannet C, Gilbert-Dussardier B, Gohler C, Goizet C, Journel H, Mom T, Thuillier-Obstoy MF, Couderc R, Garabédian EN, Denoyelle F, Marlin S. Screening of SLC26A4, FOXI1 and KCNJ10 genes in unilateral hearing impairment with ipsilateral enlarged vestibular aqueduct. Int J Pediatr Otorhinolaryngol. 2010; 74:1049–1053. [PubMed: 20621367]

Katz B. Les constantes electriques de la membrane du muscle. Arch Sci Physiol. 1949; 3:285–299.

Kelly JJ, Forge A, Jagger DJ. Development of gap junctional intercellular communication within the lateral wall of the rat cochlea. Neuroscience. 2011; 180:360–369. [PubMed: 21320575]

Kikuchi T, Kimura RS, Paul DL, Adams JC. Gap junctions in the rat cochlea: Immunohistochemical and ultrastructural analysis. Anat Embryol. 1995; 191:101–118. [PubMed: 7726389]

Kofuji P, Ceelen P, Zahs KR, Surbeck LW, Lester HA, Newman EA. Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. J Neurosci. 2000; 20:5733–5740. [PubMed: 10908613]

Kofuji P, Biedermann B, Siddharthan V, Raap M, Iandiev I, Milenkovic I, Thomzig A, Veh RW, Bringmann A, Reichenbach A. Kir potassium channel subunit expression in retinal glial cells: implications for spatial potassium buffering. Glia. 2002; 39:292–303. [PubMed: 12203395]

Kurachi Y. Voltage-dependent activation of the inward-rectifier potassium channel in the ventricular cell membrane of guinea-pig heart. J Physiol. 1985; 366:365–385. [PubMed: 2414434]

Lachheb S, Cluzeaud F, Bens M, Genete M, Hibino H, Lourdel S, Kurachi Y, Vandewalle A, Teulon J, Paulais M. Kir4.1/Kir5.1 channel forms the major K^+ channel in the basolateral membrane of mouse renal collecting duct principal cells. Am J Physiol Renal Physiol. 2008; 294:F1398–F1407. [PubMed: 18367659]

Landa P, Differ AM, Rajput K, Jenkins L, Bitner-Glindzicz M. Lack of significant association between mutations of KCNJ10 or FOXI1 and SLC26A4 mutations in Pendred syndrome/enlarged vestibular aqueducts. BMC Med Genet. 2013; 14:85. [PubMed: 23965030]

Lautermann J, ten Cate WJ, Altenhoff P, Grummer R, Traub O, Frank H, Jahnke K, Winterhager E. Expression of the gap-junction connexins 26 and 30 in the rat cochlea. Cell Tissue Res. 1998; 294:415–420. [PubMed: 9799458]

Lewis DL, Ikeda SR, Aryee D, Joho RH. Expression of an inwardly rectifying K^+ channel from rat basophilic leukemia cell mRNA in Xenopus oocytes. FEBS Lett. 1991; 290:17–21. [PubMed: 1915870]

Liu YP, Zhao HB. Cellular characterization of Connexin26 and Connnexin30 expression in the cochlear lateral wall. Cell Tissue Res. 2008; 333:395–403. [PubMed: 18581144]

Lorenz JN, Baird NR, Judd LM, Noonan WT, Andringa A, Doetschman T, Manning PA, Liu LH, Miller ML, Shull GE. Impaired renal NaCl absorption in mice lacking the ROMK potassium channel, a model for type II Bartter's syndrome. J Biol Chem. 2002; 277:37871–37880. [PubMed: 12122007]

Lourdel S, Paulais M, Cluzeaud F, Bens M, Tanemoto M, Kurachi Y, Vandewalle A, Teulon J. An inward rectifier K^+ channel at the basolateral membrane of the mouse distal convoluted tubule: similarities with Kir4–Kir5.1 heteromeric channels. J Physiol. 2002; 538:391–404. [PubMed: 11790808]

Marcus DC, Rokugo M, Thalmann R. Effects of barium and ion substitutions in artificial blood on endocochlear potential. Hear Res. 1985; 17:79–86. [PubMed: 3997684]

Marcus DC, Wu T, Wangemann P, Kofuji P. *KCNJ10* (Kir4.1) potassium channel knockout abolishes endocochlear potential. Am J Physiol Cell Physiol. 2002; 282:C403–C407. [PubMed: 11788352]

McKinney LC, Gallin EK. Inwardly rectifying whole-cell and single-channel K currents in the murine macrophage cell line J774.1. J Membr Biol. 1988; 103:41–53. [PubMed: 3184170]

Melichar I, Syka J. Electrophysiological measurements of the stria vascularis potentials *in vivo*. Hear Res. 1987; 25:35–43. [PubMed: 2433260]

Mikaelian D, Ruben RJ. Development of hearing in the normal CBA-J mouse. Acta Otol. Laryngol. 1965; 59:451–461.

Neusch C, Rozengurt N, Jacobs RE, Lester HA, Kofuji P. Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and in vivo myelination. J Neurosci. 2001; 21:5429–5438. [PubMed: 11466414]

Newman EA. Regional specialization of retinal glial cell membrane. Nature. 1984; 309:155–157. [PubMed: 6717594]

Nichols CG, Lopatin AN. Inward rectifier potassium channels. Ann Rev Physiol. 1997; 59:171–191. [PubMed: 9074760]

Nin F, Hibino H, Doi K, Suzuki T, Hisa Y, Kurachi Y. The endocochlear potential depends on two K^+ diffusion potentials and an electrical barrier in the stria vascularis of the inner ear. Proc Natl Acad Sci USA. 2008; 105:1751–1756. [PubMed: 18218777]

Nobles M, Benians A, Tinker A. Heterotrimeric G proteins precouple with G protein-coupled receptors in living cells. Proc Natl Acad Sci USA. 2005; 102:18706–18711. [PubMed: 16352729]

North RA, Williams JT, Surprenant A, Christie MJ. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. Proc Natl Acad Sci USA. 1987; 84:5487–5491. [PubMed: 2440052]

Parrock S, Hussain S, Issler N, Differ AM, Lench N, Guarino S, Oosterveld MJ, Keijzer-Veen M, Brilstra E, van Wieringen H, Konijnenberg AY, Amin-Rasip S, Dumitriu S, Klootwijk E, Knoers N, Bockenhauer D, Kleta R, Zdebik AA. KCNJ10 mutations display differential sensitivity to heteromerisation with KCNJ16. Nephron Physiol. 2013; 123:7–14. [PubMed: 24193250]

Reichold M, Zdebik AA, Lieberer E, Rapedius M, Schmidt K, Bandulik S, Sterner C, Tegtmeier I, Penton D, Baukrowitz T, Hulton SA, Witzgall R, Ben-Zeev B, Howie AJ, Kleta R, Bockenhauer D, Warth R. KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. Proc Natl Acad Sci USA. 2010; 107:14490–14495. [PubMed: 20651251]

Rosenbluth J. The fine structure of acoustic ganglia in the rat. J Cell Biol. 1962; 12:329–359. [PubMed: 14493992]

Rozengurt N, Lopez I, Chiu CS, Kofuji P, Lester HA, Neusch C. Time course of inner ear degeneration and deafness in mice lacking the Kir4.1 potassium channel subunit. Hear Res. 2003; 177:71–80. [PubMed: 12618319]

Ruppersberg JP. Intracellular regulation of inward rectifier K^+ channels. Pflugers Arch. 2000; 441:1–11. [PubMed: 11205046]

Ryan AF, Schwartz IR. Preferential amino acid uptake identifies Type II spiral ganglion neurons in the gerbil. Hear Res. 1983; 9:173–184. [PubMed: 6833161]

Sadanaga M, Morimitsu T. Development of endocochlear potential and its negative component in mouse cochlea. Hear Res. 1995; 89:155–161. [PubMed: 8600121]

Sakaguchi N, Crouch JJ, Lytle C, Schulte BA. Na-K-Cl cotransporter expression in the developing and senescent gerbil cochlea. Hear Res. 1998; 118:114–122. [PubMed: 9606066]

Sala-Rabanal M, Kucheryavykh LY, Skatchkov SN, Eaton MJ, Nichols CG. Molecular mechanisms of EAST/SeSAME syndrome mutations in Kir4.1 (KCNJ10). J Biol Chem. 2010; 285:36040–36048. [PubMed: 20807765]

Salt AN, Melichar I, Thalmann R. Mechanisms of endocochlear potential generation by stria vascularis. Laryngoscope. 1987; 97:984–991. [PubMed: 3613802]

Scholl UI, Choi M, Liu T, Ramaekers VT, Häusler MG, Grimmer J, Tobe SW, Farhi A, Nelson-Williams C, Lifton RP. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in KCNJ10. Proc Natl Acad Sci USA. 2009; 106:5842–5847. [PubMed: 19289823]

Scholl UI, Dave HB, Lu M, Farhi A, Nelson-Williams C, Listman JA, Lifton RP. SeSAME/EAST syndrome--phenotypic variability and delayed activity of the distal convoluted tubule. Pediatr Nephrol. 2012; 27:2081–2090. [PubMed: 22907601]

Schulte BA, Adams JC. Distribution of immunoreactive Na^+,K^+-ATPase in gerbil cochlea. J Histochem Cytochem. 1989; 37:127–134. [PubMed: 2536055]

Schulte BA, Steel KP. Expression of alpha and beta subunit isoforms of Na, K-ATPase in the mouse inner ear and changes with mutations at the Wv or Sld loci. Hear Res. 1994; 78:65–76. [PubMed: 7961179]

Seifert G, Hüttmann K, Binder DK, Hartmann C, Wyczynski A, Neusch C, Steinhäuser C. Analysis of Astroglial K^+ Channel Expression in the Developing Hippocampus Reveals a Predominant Role of the Kir4.1 Subunit. J Neurosci. 2009; 29:7474–7488. [PubMed: 19515915]

Shieh CC, Coghlan M, Sullivan JP, Gopalakrishnan M. Potassium channels: Molecular defects, diseases, and therapeutic opportunities. Pharmacol Rev. 2000; 52:557–594. [PubMed: 11121510]

Shuck ME, Piser TM, Bock JH, Slightom JL, Lee KS, Bienkowski MJ. Cloning and characterization of two K^+ inward rectifier (Kir) 1.1 potassium channel homologs from human kidney (Kir1.2 and Kir1.3). J Biol Chem. 1997; 272:586–593. [PubMed: 8995301]

Silver MR, DeCoursey TE. Intrinsic gating of inward rectifier in bovine pulmonary artery endothelial cells in the presence or absence of internal $Mg2^+$ J Gen Physiol. 1990; 96:109–133. [PubMed: 2212977]

Sims SM, Dixon SJ. Inwardly rectifying K current in osteoclasts. Am J Physiol Cell Physiol. 1989; 256:C1277–C1282.

Singh R, Wangemann P. Free radical stress-mediated loss of *Kcnj10* protein expression in stria vascularis contributes to deafness in Pendred syndrome mouse model. Am J Physiol Renal Physiol. 2008; 294:F139–F148. [PubMed: 17959752]

Sobkowicz, HM. The development of innervation in the organ of Corti. In: Romand, R., editor. Development of Auditory and Vestibular Systems 2. Amsterdam: Elsevier; 1992. p. 59-100.

Song MH, Shin JW, Park HJ, Lee KA, Kim Y, Kim UK, Jeon JH, Choi JY. Intrafamilial phenotypic variability in families with biallelic SLC26A4 mutations. Laryngoscope. 2013 [Epub ahead of print].

Stanfield PR, Nakajima S, Nakajima Y. Constitutively active and G-protein coupled inward rectifier K^+ channels: Kir2.0 and Kir3.0. Rev Physiol Biochem Pharmacol. 2002; 145:47–79. [PubMed: 12224528]

Takahashi T. Inward rectification in neonatal rat spinal motoneurones. J Physiol. 1990; 423:47–62. [PubMed: 2388157]

Takeuchi S, Ando M. Inwardly rectifying K^+ currents in intermediate cells in the cochlea of gerbils: a possible contribution to the endocochlear potential. Neurosci Lett. 1998; 247:175–178. [PubMed: 9655621]

Takeuchi S, Ando M, Kakigi A. Mechanism generating endocochlear potential: role played by intermediate cells in stria vascularis. Biophys J. 2000; 79:2572–2582. [PubMed: 11053131]

Takeuchi S, Ando M, Sato T, Kakigi A. Three-dimensional and ultrastructural relationships between intermediate cells and capillaries in the gerbil stria vascularis. Hear Res. 2001; 155:103–112. [PubMed: 11335080]

Takeuchi S, Kakigi A, Takeda T, Saito H, Irimajiri A. Intravascularly applied K-channel blockers suppress differently the positive endocochlear potential maintained by vascular perfusion. Hear Res. 1996; 101:181–185. [PubMed: 8951443]

Takumi T, Ishii T, Horio Y, Morishige K, Takahashi N, Yamada M, Yamashita T, Kiyama H, Sohmiya K, Nakanishi S, Kurachi Y. A novel ATP-dependent inward rectifier potassium channel expressed predominantly in glial cells. J Biol Chem. 1995; 270:16339–16346. [PubMed: 7608203]

Tang X, Hang D, Sand A, Kofuji P. Variable loss of Kir4.1 channel function in SeSAME syndrome mutations. Biochem Biophys Res Commun. 2010; 399:537–541. [PubMed: 20678478]

Tasaki I, Spyropoulos CS. Stria vascularis as source of endocochlear potential. J Neurophysiol. 1959; 22:149–155. [PubMed: 13642091]

ten Cate WJ, Curtis LM, Rarey KE. Na,K-ATPase alpha and beta subunit isoform distribution in the rat cochlear and vestibular tissues. Hear Res. 1994; 75:151–160. [PubMed: 8071142]

Thompson DA, Feather S, Stanescu HC, Freudenthal B, Zdebik AA, Warth R, Ognjanovic M, Hulton SA, Wassmer E, van't Hoff W, Russell-Eggitt I, Dobbie A, Sheridan E, Kleta R, Bockenhauer D. Altered electroretinograms in patients with KCNJ10 mutations and EAST syndrome. J Physiol. 2011; 589:1681–1689. [PubMed: 21300747]

Wang XH, Streeter M, Liu YP, Zhao HB. Identification and characterization of pannexin expression in the mammalian cochlea. J Comp Neurol. 2009; 512:336–346. [PubMed: 19009624]

Wangemann P. Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential. J Physiol. 2006; 576:11–21. [PubMed: 16857713]

Wangemann P, Itza EM, Albrecht B, Wu T, Jabba SV, Maganti RJ, Lee JH, Everett LA, Wall SM, Royaux IE, Green ED, Marcus DC. Loss of *KCNJ10* protein expression abolishes endocochlear potential and causes deafness in Pendred syndrome mouse model. BMC Med. 2004; 2:30. [PubMed: 15320950]

Wangemann, P.; Schacht, J. Homeostatic mechanisms in the cochlea. In: Dallos, P.; Popper, AN.; Fay, RR., editors. The Cochlea. New York: Springer; 1996. p. 130-185.

Williams DM, Lopes CM, Rosenhouse-Dantsker A, Connelly HL, Matavel A, O-Uchi J, McBeath E, Gray DA. Molecular basis of decreased Kir4.1 function in SeSAME/EAST syndrome. J Am Soc Nephrol. 2010; 21:2117–2129. [PubMed: 21088294]

Yang H, Xiong H, Huang Q, Pang J, Zheng X, Chen L, Yu R, Zheng Y. Compromised potassium recycling in the cochlea contributes to conservation of endocochlear potential in a mouse model of age-related hearing loss. Neurosci Lett. 2013; 555:97–101. [PubMed: 24055606]

Yang T, Gurrola JG 2nd, Wu H, Chiu SM, Wangemann P, Snyder PM, Smith RJ. Mutations of *KCNJ10* together with mutations of SLC26A4 cause digenic nonsyndromic hearing loss associated with enlarged vestibular aqueduct syndrome. Am J Hum Genet. 2009; 84:651–657. [PubMed: 19426954]

Highlights

- Kir4.1 (*KCNJ10*) is required for EP and spiral ganglion function in inner ear
- Deficiency of Kir4.1 causes deafness with EAST or SeSAME syndromes
- Deafness mutants show “loss of function” and can be rescued by WT isoform
- Deafness mechanisms of Kir4.1 deficiency are discussed and prospected

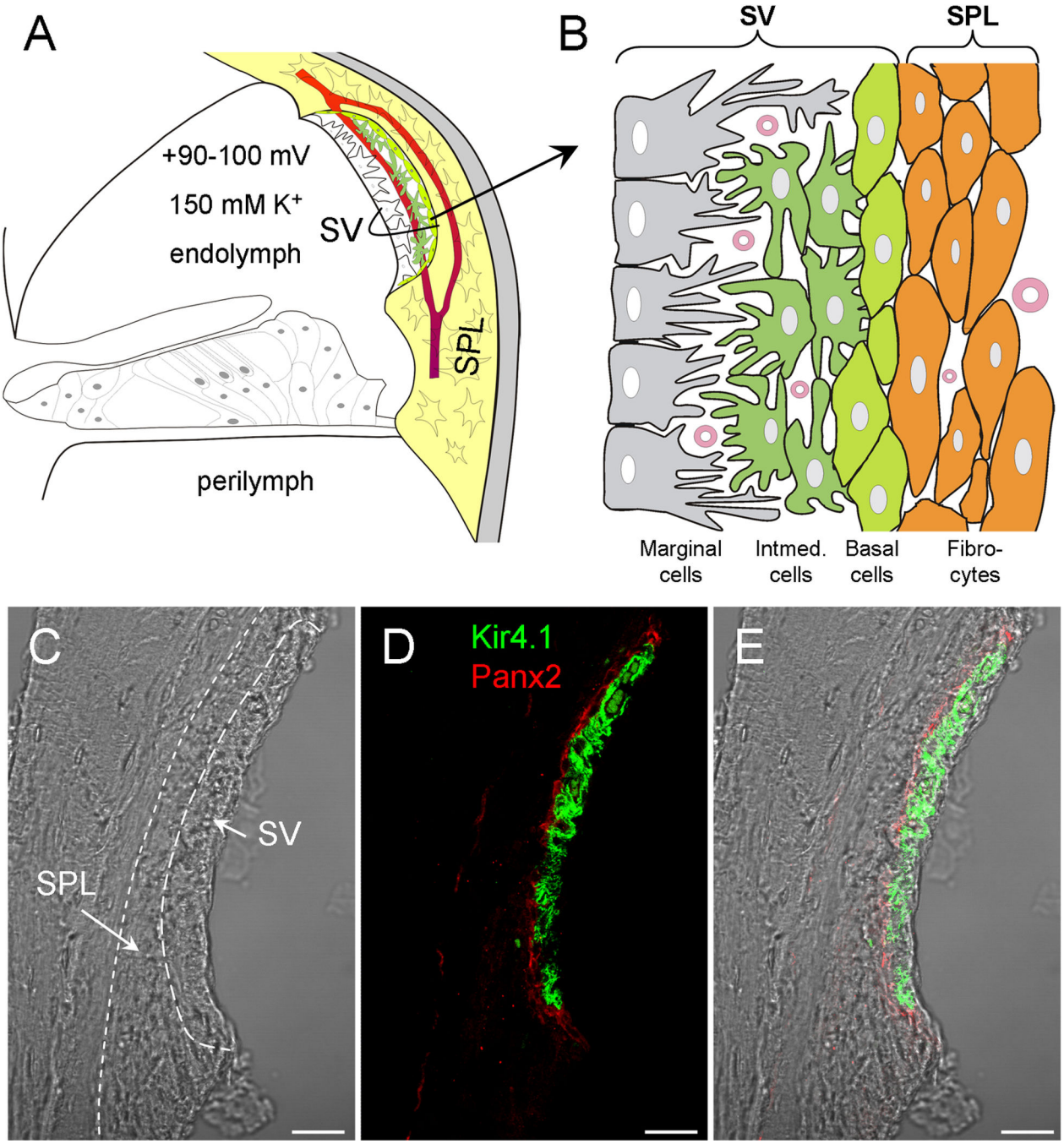

Fig. 1.

Expression of Kir4.1 in the cochlear lateral wall. A–B: Schematic drawing of the cochlea and the cochlear lateral wall. C-E: Kir4.1 labeling in the intermediate cells in the cochlear lateral wall. The basal cells are visible by Pannexin2 (Panx2) labeling (red) (Wang et al., 2009). Note that the basal cells have no Kir4.1 labeling (green). SV: stria vascularis; SPL: spiral ligament. Scale bar: 25 μm.

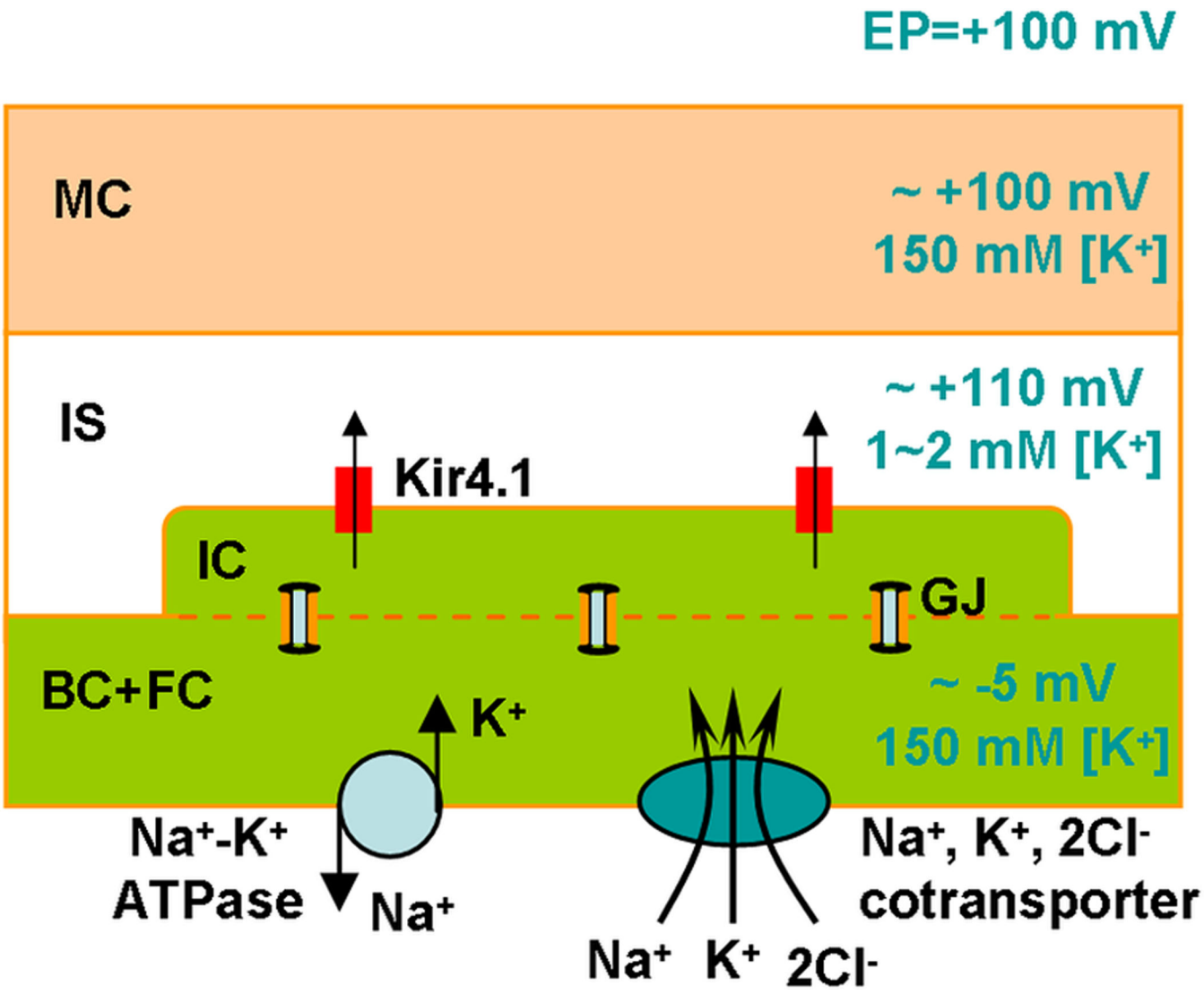

Fig. 2.

Schematic drawing of “two-cell” model for EP generation. Intermediate cells (IC), basal cells (BC) and fibrocytes (FC) are coupled by gap junctions (GJ) acting as one cell. Marginal cells (MC) act as another cell. Na^+, K^+-ATPase and Na^+, K^+, $2Cl^-$-cotransporter are located at the bottom of the FCs to pump K^+ ions into cells leading to cells depolarizing. Kir4.1 is located at the apical membrane of the ICs facing to the intrastrial space (IS) to generate positive EP.

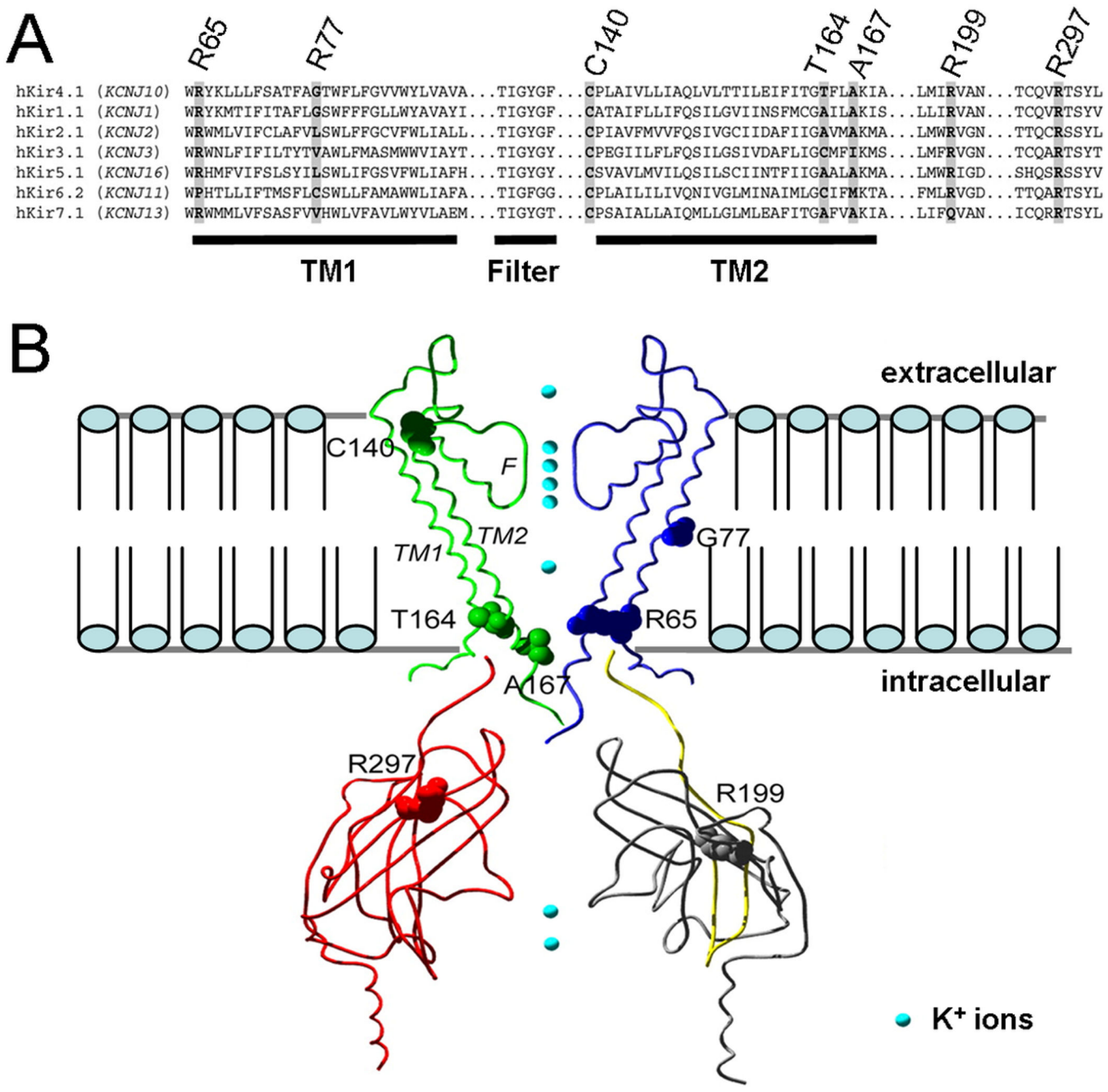

Fig. 3.

Predicted structure of the Kir4.1 channel and locations of mutations. A: Sequence alignment of human Kir4.1 (P78508.1) with hKir1.1 (P48048.1), hKir2.1 (P63252.1), hKir3.1 (P48549.1), hKir5.1 (Q9NPI9.1), hKir6.2 (Q14654.2), and hKir7.1 (O60928.1). B: A Kir4.1 subunit consists of two transmembrane domains (TM1 and TM2) that flank a K^+-selective pore (F). A functional channel is assembled by 4 subunits. Only two are displayed for clarity. Modified from Sala-Rabanal et al., 2010.