Müller cells upregulate the expression of SAP97 in light-injured rat retina

Hui Ren[a], Gezhi Xu[a,b], Jiang Qian[a], Min Zhou[a], John Gonzales[c] and Yingqin Ni[a]

SAP97 is thought to play key roles in synapse assembly and synaptic plasticity. This study was carried out to determine whether it is involved in the Müller cell response to blue light injury. In light-injured rats, obvious intracellular edema in the outer retina was observed by transmission electron microscopy. The immunostaining of SAP97 was upregulated and concentrated in the Müller cell processes after photic injury, which was similar to the changes of AQP4 and the inwardly rectifying potassium channel, Kir4.1. Western blots showed that SAP97 and AQP4 protein levels were both increased on the third day after light exposure when compared with the control group ($P<0.05$). The upregulation of SAP97 coincides with the redistribution of AQP4 and Kir4.1 in blue light-injured rat retina. *NeuroReport* **21:832–836 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

NeuroReport 2010, **21**:832–836

Keywords: aquaporin, blue light injury, Kir4.1, Müller cell, outer retinal edema, SAP97

[a]EENT Hospital, Eye Institute, [b]Institute of Brain Science, Fudan University, Shanghai, China and [c]Department of Ophthalmology and Visual Sciences, University of Texas Medical Branch, Galveston, Texas, USA

Correspondence to Dr Gezhi Xu, EENT Hospital, Eye Institute, Fudan University, Fengyang Road 83, Shanghai 200031, China
Tel: +86 21 64377134; fax: +86 21 64376491;
e-mail: ophthalmology.retina@gmail.com

Received 1 April 2010 **accepted** 15 June 2010

Introduction

Retinal edema is one of the leading causes of visual impairment in patients with uveitis and diabetes. The accumulation of fluid within the retina leads to compression of neurons, nerve fibers, and vessels (which exacerbates ischemic conditions), in addition to retarding the diffusion of metabolic substrates and oxygen [1], thereby contributing to the degeneration of retinal neurons and, ultimately, leading to a decrease in visual acuity. The absorption of fluid from the subretinal space and retinal tissue into the blood is normally carried out by pigment epithelial and glial cells through transcellular water transport coupled to currents through potassium channels in Müller cells [2]. Earlier studies have shown that retinal edema is accompanied with an alteration in the expression of Kir4.1 and AQP4 of Müller cells [3,4]. The precise pathogenic mechanisms underlying the alteration of these channels are still not fully understood.

The synaptic membrane-associated guanylate kinase scaffolding protein family consists of four mammalian homologs: SAP90/PSD95 [5,6], SAP97/Hdlg [7,8], PSD93/Chapsyn-110 [9,10], and SAP102 [11], and are thought to play key roles in synapse assembly and synaptic plasticity. A study of the rat hippocampal slice cultures showed that increasing the level of synaptic PSD-95 recruits new synaptic-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors to synapses without changing the number of surface amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors [12]. Inward rectifier potassium (Kir) channels, including Kir2.1, Kir2.2, Kir2.3, and Kir4.1, were associated with scaffolding proteins, such as SAP97, PSD95/SAP90, and CASK through the C-terminal postsynaptic density protein-95, disk-large tumor suppressor protein, zonula occludens-1binding motif [13–15]. However, it is not known whether alteration of the expression of Kir4.1 and AQP4 in Müller cells under pathological conditions, such as diabetic retinopathy and light injury to the retina, is associated with the changes of membrane-associated guanylate kinase protein family members such as SAP97.

The purpose of this study was to determine whether the distribution and expression of scaffolding protein, SAP97, is associated with the alteration of Kir4.1 and AQP4 in Müller cells when outer retinal edema is induced in light-injured rat retina.

Materials and methods

Animals and light exposure

All procedures were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Adult Sprague–Dawley rats weighing 190–210 g were used. Light damage in the retinas of Sprague–Dawley rats was induced as described earlier [16]. The animals were anesthetized by intramuscular injection of ketamine (120 mg/kg) and xylazine (6 mg/kg) during examination and were killed by an overdose of pentobarbitone at the end of the experiment.

Transmission electron microscopy

Anesthetized rats were transcardially perfused with 200 ml of 0.9% saline, followed by 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.4). The eyes were then enucleated and immersion-fixed in 2.5%

0959-4965 DOI: 10.1097/WNR.0b013e32833d40ab

glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) after the removal of the cornea and lens and postfixed in 1.0% osmium tetroxide. The tissues were then dehydrated in a progressive ethanol and acetone solution before being embedded in Epon812 (Serva, Heidelberg, Germany). An LKB ultramicrotome (LKB Instruments, Inc., Gaithersburg, Maryland, USA) was then used to section the tissues before being stained with uranyl acetate, which was followed by lead citrate. Prepared tissues were then observed with a Philips CM120 transmission electron microscope (Philips Inc., Eindhoven, The Netherlands).

Immunohistochemistry

Immunohistochemistry of retinal tissue sections was conducted as described earlier [16]. The following primary antibodies were used: polyclonal rabbit anti-Kir4.1 (1 : 300; Alomone Laboratories, Jerusalem, Israel); monoclonal mouse antirat SAP97 (1 : 300; StressGen, Victoria, British Columbia, Canada); monoclonal mouse antirat glial fibrillary acidic protein (GFAP) (1 : 500; Thermo/Abcom, Cambridge, Massachusetts, USA); rabbit antimouse glutamine synthetase (1 : 2000; Chemicon, Temecula, California, USA); and rabbit anti-AQP4 (1 : 600; Sigma, Sigma-Aldrich, St Louis, Missouri, USA). The secondary antibodies were AlexaFluor 555 goat antimouse immunoglobulin G (1 : 800; Molecular Probe, Eugene, Oregon, USA) and AlexaFluor 488 goat antirabbit immunoglobulin G (1 : 800; Molecular Probe). Nuclei staining was achieved with 4′,6-diamidino-2-phenylindole (1 : 500; Chemicon). Retinal sections were examined under a confocal laser microscope (Olympus Fluoview FV1000, Olympus, Tokyo, Japan) and the color saturation of the fluorescence microscope images was enhanced by the same amount in Olympus Fluoview Ver.1.7b Viewer (Olympus).

Western blot analysis

Western blot was carried out as described earlier [16].

Statistical analysis

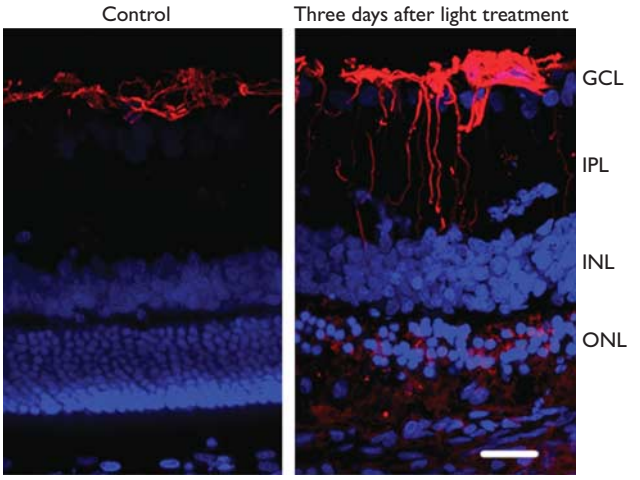
All the experiments were performed at least three times. Mixed model type 3 tests of fixed effects were used to assess the differences among the groups. *P* values of less than 0.05 were considered significant.

Results

Our earlier study showed that excessive light induces significant loss of photoreceptor cells [16]. In this study, transmission electron micrographs showed that inner and outer segments of photoreceptors were aligned uniformly with some space between one another in the control retina. The inner segments of photoreceptors were markedly swollen on day 3.

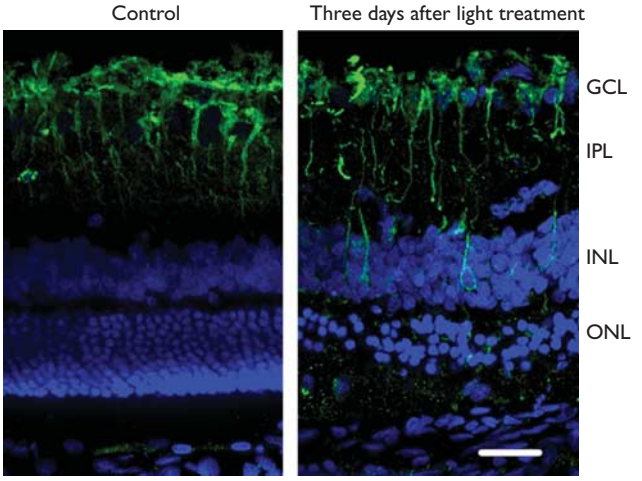
Three days after blue light exposure, a significant increase of GFAP immunoreactivity in Müller cells was observed (Fig. 1). Immunoreactivity of both Kir4.1 (Fig. 2) and AQP4 (Fig. 3c) emerged in the outer nuclear layer in the light-injured retina. Scaffolding protein, SAP97, which was predominantly expressed and costained with AQP4 in the outer plexiform layer (Figs 3b and 4), exhibited increased immunoreactivity in the outer nuclear layer (Fig. 4). SAP97 was costained with Müller cell marker glutamine synthetase around the photoreceptor cells after light exposure (Fig. 4). Levels of SAP97 protein were increased on days

Fig. 1



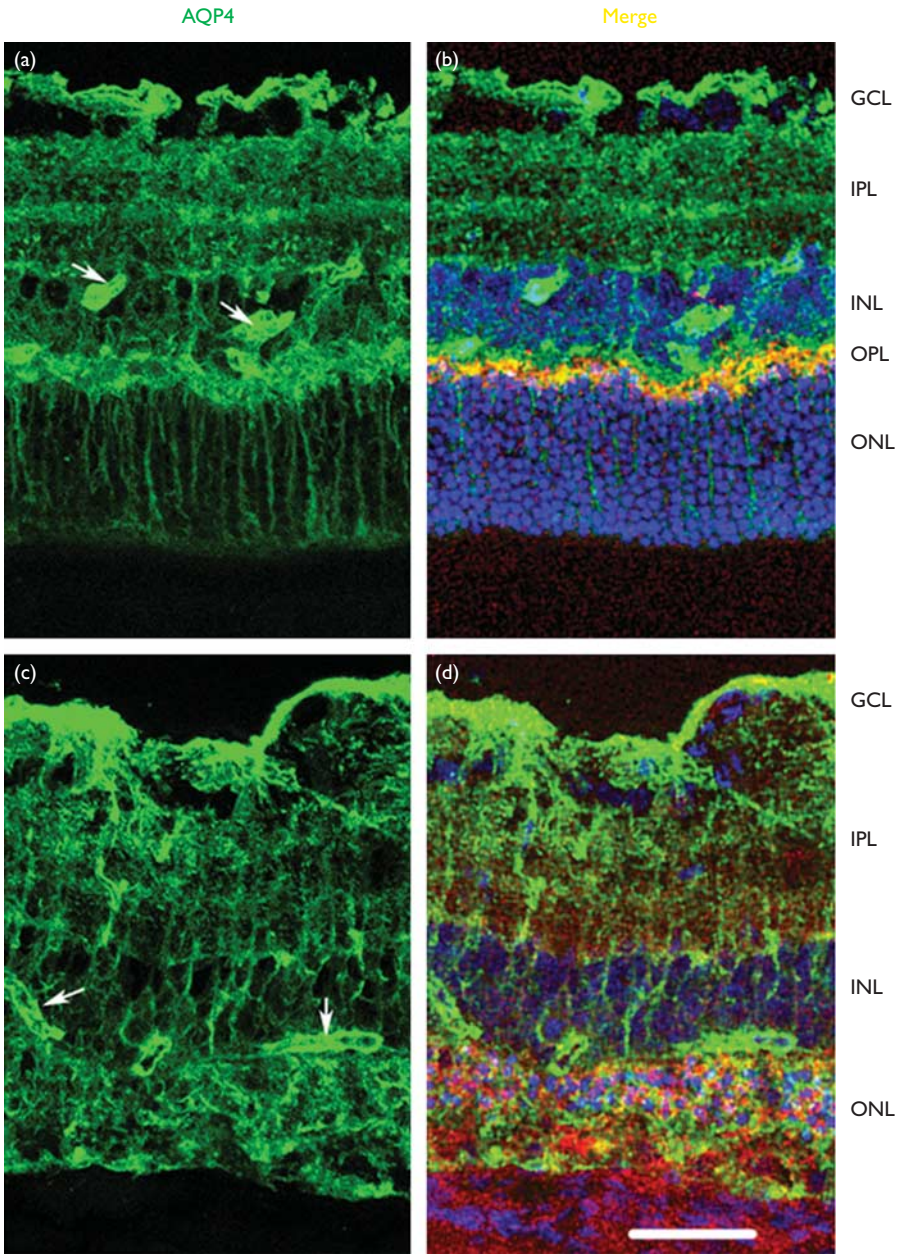
Immunostaining against the glial cell marker glial fibrillary acidic protein (GFAP). In control tissues, astrocytes in the ganglion cell layer (GCL) display GFAP labeling. Three days after the blue light exposure, Müller cell fibers express GFAP immunoreactivity. Scale bar = 20 μm. INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer.

Fig. 2



Staining of the inwardly rectifying potassium channel (Kir4.1). After the light treatment, Kir4.1 emerged in the outer nuclear layer. Scale bar = 20 μm. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer.

Fig. 3



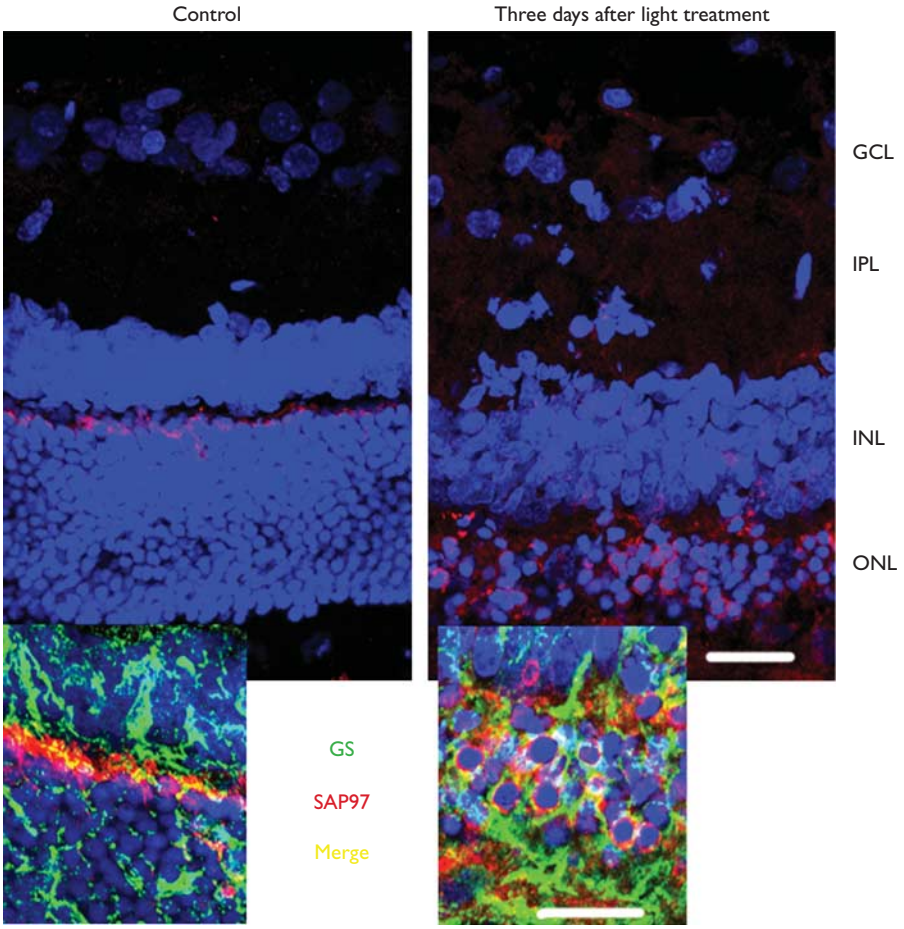
Double immunofluorescence for SAP97 (red) and AQP4 (green) in both control and 3 days after the light-exposure of the rat retina. In the control retina (a and b), AQP4 was mainly stained in the inner limiting membrane and around the vessels (white arrows) and was costained with SAP97 in the outer plexiform layer (OPL) (b). On the third day after the light treatment, AQP4 emerged in the outer nuclear layer (ONL) (c). Similarly, SAP97 exhibited increased immunoreactivity in the ONL and it was colocalized with AQP4 in the ONL (d). A thinner ONL was found in the light-injured retina (d) compared with the control group. Scale bar = 20 μm. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer.

2 and 3 ($P < 0.05$) followed by an increase of AQP4 protein level on day 3 ($P < 0.05$), whereas there was no statistically significant alteration in the Kir4.1 protein level (data not shown).

Discussion

In this study, we observed that the blue light-injured rat retina exhibited intracellular edema in the outer retina using transmission electron micrographs. In this animal model of acute outer retinal edema and photoreceptor degeneration, Müller cells displayed activation as noted by an increase in GFAP expression with subsequent emergence in the expression of both Kir4.1 and AQP4 from the inner retina to the outer retina. These results are in accordance with those from the studies carried out by Iandiev *et al.* [3,4]. The simultaneous increase in the

Fig. 4



Staining against SAP97. The insets display staining against SAP97 (red) and costaining of SAP97 and the glial cell marker glutamine synthetase (green) (down). The yellow merge signal indicates that Müller glial cells express SAP97 around photoreceptor cells after light exposure. Scale bar = 20 μm. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer.

immunolabeling of AQP4 and Kir4.1 channel proteins in the outer retina suggests a functional adaptation to a pathological situation.

SAP97 is a member of the scaffolding protein family, which contains three N-terminal postsynaptic density protein-95, disk-large tumor suppressor protein, zonula occludens-1 domains, an Src-homology 3 domain, and a C-terminal inactive guanylate kinase domain [17,18]. Each of these domains is a site of protein–protein interaction and seems to play a role in the localization of ligand-gated and/or voltage-gated ion channels and adhesion molecules in addition to the assembly of synaptic junctions [19]. Earlier studies have shown that SAP97 can affect the synaptic recruitment of many postsynaptic proteins, such as amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, Kv4.2 potassium channel, glutamate receptor 1 [20–22], and Kir4.1 was colocalized with SAP97 on the cell membranes of isolated Müller cells of Wistar rats [23]. Furthermore, both Kir4.1 and AQP4 were tethered together in Müller cells [23,24]. These studies show a close association between SAP97 and ion channels, such as AQP4 and Kir4.1, in normal conditions. For the first time, our results indicate that an enhanced expression of SAP97 in the outer retina of light-damaged retinas was consistent with alterations in AQP4 and Kir4.1. We propose that after light injury, in response to outer retinal edema and excessive efflux of ions from dying photoreceptor cells, Müller cells upregulate the expression of SAP97 and AQP4. Increased expression of SAP97 in the Müller cell processes may facilitate both AQP4 and Kir4.1 to redistribute to the outer nuclear layer of the retina. To establish this point definitively, however, more biochemical experiments will be required.

Conclusion

Our results indicate that, in a model of photic injury, Müller cells become activated and upregulate the expression of SAP97, which became evident in the outer retina along with a similar redistribution of Kir4.1 and AQP4.

Acknowledgements

This work was supported by grants from the National Basic Research Program of China (973 program), (2007 CB512205), National Basic Research Grants of China (30872825, 2008), and Plan of the Best Disciplines Leaders in Shanghai (09XD1400900).

References

1 Reichenbach A, Wurm A, Pannicke T, Iandiev I, Wiedemann P, Bringmann A. Müller cells as players in retinal degeneration and edema. *Graefe's Arch Clin Exp Ophthalmol* 2007; **245**:627–636.
2 Nagelhus EA, Horio Y, Inanobe A, Fujita A, Haug FM, Nielsen S, *et al.* Immunogold evidence suggests that coupling of K siphoning and water transport in rat retinal Müller cells is mediated by a coenrichment of Kir4.1 and AQP4 in specific membrane domains. *Glia* 1999; **26**:47–54.
3 Iandiev I, Pannicke T, Hollborn M, Wiedemann P, Reichenbach A, Grimm C, *et al.* Localization of glial aquaporin-4 and Kir4.1 in the light-injured murine retina. *Neurosci Lett* 2008; **434**:317–321.
4 Iandiev I, Wurm A, Hollborn M, Wiedemann P, Grimm C, Remé CE, *et al.* Müller cell response to blue light injury of the rat retina. *Invest Ophthalmol Vis Sci* 2008; **49**:3559–3567.
5 Cho KO, Hunt CA, Kennedy MB. The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. *Neuron* 1992; **9**:929–942.
6 Kistner U, Wenzel BM, Veh RW, Cases-Langhoff C, Garner AM, Appeltauer U, *et al.* SAP90, a rat presynaptic protein related to the product of the Drosophila tumor suppressor gene dlg-A. *J Biol Chem* 1993; **268**:4580–4583.
7 Lue RA, Marfatia SM, Branton D, Chishti AH. Cloning and characterization of hdlg: the human homologue of the Drosophila discs large tumor suppressor binds to protein 4.1. *Proc Natl Acad Sci U S A* 1994; **91**:9818–9822.
8 Muller BM, Kistner U, Veh RW, Cases-Langhoff C, Becker B, Gundelfinger ED, *et al.* Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the Drosophila discs-large tumor suppressor protein. *J Neurosci* 1995; **15**:2354–2366.
9 Brenman JE, Christopherson KS, Craven SE, McGee AW, Bredt DS. Cloning and characterization of postsynaptic density 93, a nitric oxide synthase interacting protein. *J Neurosci* 1996; **16**:7407–7415.
10 Kim E, Cho KO, Rothschild A, Sheng M. Heteromultimerization and NMDA receptor-clustering activity of Chapsyn-110, a member of the PSD-95 family of proteins. *Neuron* 1996; **17**:103–113.
11 Muller BM, Kistner U, Kindler S, Chung WJ, Kuhlendahl S, Fenster SD, *et al.* SAP102, a novel postsynaptic protein that interacts with NMDA receptor complexes *in vivo*. *Neuron* 1996; **17**:255–265.
12 Schnell E, Sizemore M, Karimzadegan S, Chen L, Bredt DS, Nicoll RA. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc Natl Acad Sci U S A* 2002; **99**:13902–13907.
13 Leonoudakis D, Conti LR, Anderson S, Radeke CM, McGuire LMM, Adams ME, *et al.* Protein trafficking and anchoring complexes revealed by proteomic analysis of inward rectifier potassium channel (Kir2.x)-associated proteins. *J Biol Chem* 2004; **279**:22331–22346.
14 Nehring RB, Wischmeyer E, Döring F, Veh RW, Sheng M, Karschin A. Neuronal inwardly rectifying K1 channels differentially couple to PDZ proteins of the PSD-95/SAP90 family. *J Neurosci* 2000; **20**:156–162.
15 Goult BT, Rapley JD, Dart C, Kitmitto A, Grossmann JG, Leyland ML, *et al.* Small-angle x-ray scattering and NMR studies of the conformation of the PDZ region of SAP97 and its interactions with Kir2.1. *Biochemistry* 2007; **46**:14117–14128.
16 Ni YQ, Xu GZ, Hu WZ, Shi L, Qin YW, Da CD. Neuroprotective effects of naloxone against light-induced photorecepor degeneration through inhibiting retinal microglial activation. *Invest Ophthalmol Vis Sci* 2008; **49**:2589–2598.
17 Garner CC, Kindler S. Synaptic proteins and the assembly of synaptic junctions. *Trends Cell Biol* 1996; **6**:429–433.
18 Kuhlendahl S, Konrad M, Kim E, Garner CC, Spangenberg O. Functional analysis of the guanylate kinase-like domain in the synapse–associated protein SAP97. *Eur J Biochem* 1998; **252**:305–313.
19 Garner CC, Nash J, Huganir RL. PDZ domains in synapse assembly and signaling. *Trends Cell Biol* 2000; **10**:274–280.
20 Rumbaugh G, Sia GM, Garner CC, Huganir RL. Synapse-associated protein-97 isoform-specific regulation of surface AMPA receptors and synaptic function in cultured neurons. *J Neurosci* 2003; **23**:4567–4576.
21 Regalado MP, Terry-Lorenzo RT, Waites CL, Garner CC, Malenka RC. Transsynaptic signaling by postsynaptic synapse-associated protein 97. *J Neurosci* 2006; **26**:2343–2357.
22 Gardoni F, Mauceri D, Marcello E, Sala C, Luca MD, Jeromin A. SAP97 directs the localization of Kv4.2 to spines in hippocampal neurons regulation by CaMKII. *J Biol Chem* 2007; **282**:28691–28699.
23 Horio Y, Hibino H, Inanobe A, Yamada M, Ishii M, Tada Y, *et al.* Clustering and enhanced activity of an inwardly rectifying potassium channel, Kir4.1, by an anchoring protein, PSD-95/SAP90. *J Biol Chem* 1997; **272**:12885–12888.
24 Connors NC, Kofuji P. Potassium channel Kir4.1 macromolecular complexin retinal glial cells. *Glia* 2006; **53**:124–131.