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Gβ5–RGS complexes co-localize with mGluR6 in retinal ON-bipolar cells

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Abstract

The time course of G-protein-coupled responses is largely determined by the kinetics of GTP hydrolysis by the G protein α subunit, which is accelerated by interaction with regulator of G-protein signaling (RGS) proteins. Light responses of ON-bipolar cells of the vertebrate retina require rapid inactivation of the G protein G α_o , which is activated in the dark by metabotropic glutamate receptor, mGluR6, in their dendritic tips. It is not yet known, however, which RGS protein(s) might be responsible for rapid inactivation kinetics. By immunofluorescence and co-immunoprecipitation, we have identified complexes of the G α_o -selective RGS proteins RGS7 and RGS11, with their obligate binding partner, G β_5 , that are localized to the dendritic tips of murine rod and cone ON-bipolar cells, along with mGluR6. Experiments using pre- and post-synaptic markers, and a dissociated bipolar cell preparation, clearly identified the location of these complexes as the ON-bipolar cell dendritic tips and not the adjacent photoreceptor terminals or horizontal cell dendrites. In mice lacking mGluR6, the distribution of RGS11, RGS7 and G β_5 shifts away from the dendritic tips, implying a functional relationship with mGluR6. The precise co-localization of G β_5 –RGS7 and G β_5 –RGS11 with mGluR6, and the dependence of localization on the presence of mGluR6, suggests that G β_5 –RGS7 and G β_5 –RGS11 function specifically in the mGluR6 signal transduction pathway, where they may stimulate the GTPase activity of G α_o , thus accelerating the ON-bipolar cell light response, in a manner analogous to the acceleration of photoreceptor light responses by the G β_5 –RGS9-1 complex.

Keywords

metabotropic glutamate receptor; mouse; retina; ribbon synapse; synaptic transmission

Introduction

Glutamate release from retinal photoreceptors simultaneously depolarizes OFF-bipolar cells and hyperpolarizes ON-bipolar cells. The inhibitory action of glutamate on ON-bipolar cells is mediated by a unique metabotropic glutamate receptor, mGluR6, located in the ON-bipolar cell dendrites (Nomura *et al.*, 1994). Upon binding glutamate, mGluR6 activates a G-protein pathway that closes a non-selective cation channel, and hyperpolarizes the cell (Schiells & Falk, 1990; Nawy & Jahr, 1991; Yamashita & Wässle, 1991). In response to light, glutamate release by photoreceptors drops, and the mGluR6-coupled G α subunit, G α_o , is rapidly inactivated, causing the channels to open, thereby depolarizing the ON-bipolar cells (Nawy, 1999; Dhingra *et al.*, 2000; Vardi *et al.*, 2000). In some respects, the mGluR6 transduction pathway is similar to phototransduction, where light activates the G α subunit of transducin

(G α_T) leading to the closure of cGMP-gated cation channels and hyperpolarization of the photoreceptor.

The intrinsic rates of GTP hydrolysis by G α_o and G α_T are slow, requiring tens of seconds. In outer segments, G α_T GTPase activity is accelerated by interaction with the G β_5 L–RGS9 complex. The GTPase accelerating activity of G β_5 L–RGS9 is critical in setting the inactivation kinetics of phototransduction, as mice lacking either RGS9 (Chen *et al.*, 2000) or G β_5 (Krispel *et al.*, 2003) show normal activation of the outer segment photoresponse, but 10–50-fold slower deactivation than wild-type. It is likely that the rapid response of ON-bipolar cells to light requires a similar GTPase accelerating activity. Like G α_T , the rate of GTP hydrolysis by G α_o may be accelerated by interaction with a G β_5 –RGS complex.

Immunoreactivity for G β_5 has been reported in outer segments and the outer plexiform layer (OPL) of the retina (Zhang *et al.*, 2003). G β_5 exists as two isoforms, G β_5 S and G β_5 L, that are identical except for 42 amino acids on the amino end of G β_5 L (Watson *et al.*, 1996). The long form (G β_5 L) is retina-specific and found exclusively in outer segments (Watson *et al.*, 1996), whereas the shorter form (G β_5 S), which is present in both retina and brain, is excluded from rod outer segments, but is found in cone outer segments along with G β_5 L (Zhang *et al.*, 2003). G β_5 is the most divergent in amino acid sequence of the five known mammalian G β subunits (von Weizsacker *et al.*, 1992; Watson *et al.*, 1996). Unlike other G β subunits, which bind to G γ subunits, G β_5 forms complexes instead with the R7 family of RGS (regulator of G protein signaling) proteins that includes RGS6, RGS7, RGS9 and RGS11. The R7-RGS proteins contain a G γ -like (GGL) domain that mediates binding to G β_5 (Simonds & Zhang, 2000). They also possess a GTPase-accelerating protein (GAP) domain within a conserved ~120-amino-acid sequence (Popov *et al.*, 1997), through which they promote the intrinsic GTPase activity of G α subunits.

Here we present evidence that G β_5 –RGS7 and G β_5 –RGS11 are present in ON-bipolar cells where they co-localize with mGluR6 in the dendrites in wild-type animals, but show altered distribution in mice lacking mGluR6, suggesting a similar mechanism of deactivation of both the ON-bipolar cell response and phototransduction.

Methods

Immunohistochemistry

Immunohistochemistry on retina sections and acutely dissociated rod bipolar cells was performed as described previously (Berntson *et al.*, 2003; Morgans *et al.*, 2005). Six adult mice were used and killed by cervical dislocation in accordance with OHSU Institutional Animal Care and Use Committee guidelines. Rabbit polyclonal antibody (R4612) against full-length bovine RGS7 and goat polyclonal antibody against G β_5 (peptide MATDGLHENETLASLKC) were generated at Bethyl Laboratories (Montgomery, TX, USA). The RGS11 antibody was raised in rabbits against a recombinant polypeptide corresponding to residues 248–471 of mouse RGS11 (Chen *et al.*, 2003). The RGS9 rabbit antiserum was generated against a polypeptide corresponding to amino acids 226–484 of bovine RGS9 (He *et al.*, 1998). Antibodies were used at the following concentrations: G β_5 , 1 : 500–1 : 1000; RGS7, 1 : 500–1 : 1000; RGS9, 1 : 1000–1 : 2000; RGS11, 1 : 1000–1 : 5000; mGluR6 (Morgans *et al.*, 2006), 1 : 100; PKC α (Sigma, St Louis, MO, USA), 1 : 5000; calbindin (Sigma), 1 : 1000; anti-ctbp2/ribeye (BD Biosciences Pharmingen, San Diego, CA, USA), 1 : 5000. Appropriate secondary antibodies were coupled to either CY3 (Jackson Immunochemicals, West Grove, PA, USA) and diluted 1 : 500, or Alexa-488 (Molecular Probes, Eugene, OR, USA) and diluted 1 : 2000.

Microscopy

Images of the dissociated bipolar cells were obtained on a Nikon Eclipse E800 fluorescence microscope with a 100 $\times$ /1.30 oil-immersion objective and Metamorph software. All other images were acquired on a Zeiss LSM 510 confocal microscope with a 63 $\times$ /1.40 oil-immersion objective. All confocal figures show single optical sections of < 1 μ m thickness. For figures, brightness and contrast of images were optimized with Adobe Photoshop 7.0.

Image averaging

The localization of G β 5 relative to ribeye, calbindin and mGluR6 was determined using ImageJ software (W.S. Rasband, NIH, Bethesda, MD, USA), and a signal averaging method similar to that described by Massey & Mills (1999) and Zhang *et al.* (2002). Twenty 50 $\times$ 50-pixel images, centred on G β 5-immunopositive puncta, were averaged. The average intensity of each label was plotted using the ImageJ RGB_Profiler plugin (Laummonerie and Mutterer, Institut de Biologie Moléculaire des Plantes, Strasbourg, France).

Immunoprecipitation from mouse retina lysate

Affinity-purified anti-G β 5 or pre-immune IgG (0.6 mg) was coupled to 1 mL AffiGel-15 media (Bio-Rad) in 0.1 M 4-morpholinepropane-sulphonic acid (MOPS), pH 7.5, and used for immunoprecipitations. Freshly dissected mouse retinas were homogenized in homogenization buffer [20 mM HEPES, pH 7.0, 150 mM NaCl, 3 mM MgCl₂, 1 mM CaCl₂, 1 mM β -ME, 1 mM EDTA, 0.01% NaN₃, 0.2% C₁₂E₁₀, protease inhibitors (2 μ g/mL aprotinin, 2 μ g/mL chymostatin, 0.5 μ g/mL leupeptin, 0.7 μ g/mL pepstatin A, 30 μ g/mL trypsin inhibitor, 1.6 mg/mL benzamide, 0.1 μ M E64, 167 μ M Pefabloc, and phenylmethylsulphonyl fluoride)], sonicated on ice for 60 s and incubated at 4 $^{\circ}$ C for 1 h with gentle shaking. After centrifugation at 100 000g for 30 min, equal amounts of supernatant were applied to anti-G β 5 IgG- or pre-immune IgG-coupled columns, washed with homogenization buffer, and immunoprecipitated proteins eluted with CT215 peptide (amino-terminal 16 amino acids of G β 5) followed by SDS-PAGE and Western blotting.

Western blotting

Retinal extracts were subjected to electrophoresis on precast Novex 4–12% polyacrylamide gradient gels (Invitrogen, Carlsbad, CA, USA), and then the separated proteins electrophoretically transferred to nitrocellulose membranes, which were probed with different antibodies as previously described (Morgans *et al.*, 2006).

Results

Localization of G β 5 to ON-bipolar cell dendrites

Immunofluorescent staining of G β 5 in the mouse retina revealed punctate labeling in the OPL, and faint labeling in both the ON and the OFF strata of the inner plexiform layer (IPL) (Fig. 1A). The immunofluorescence was blocked by preincubation of the antibody with the peptide immunogen (+ pep, Fig. 1A) demonstrating specificity of the labeling. At high magnification, the OPL labeling could be resolved as pairs of smaller puncta (Fig. 1B) most likely associated with rod photoreceptor terminals. G β 5 staining associated with cone terminals was also evident (Fig. 1B). Previous work has localized the photoreceptor-specific, long isoform of G β 5, called G β 5L, to outer segments (Watson *et al.*, 1996). Despite detecting both G β 5 and G β 5L on Western blots of mouse retinal extract (Fig. 1D), we did not observe immunofluorescent staining of outer segments. The previous study used longer fixation times of 18 h in 4% paraformaldehyde, whereas we fixed for only 15 min. We found that with long fixation times (18 h), staining of outer segments increased dramatically, whereas the OPL staining markedly diminished (Fig. 1C); thus, the two G β 5 isoforms in the retina, G β 5L and G β 5, may be

differentially affected by fixation. Light fixation conditions were chosen for analysis of G β 5 in the OPL.

To compare the localization of G β 5 with ON-bipolar cell dendrites, retina sections were double labeled for G β 5 and for either G α o (present in all ON-bipolar cells) or PKC α (a rod bipolar cell marker) (Haverkamp & Wässle, 2000). As observed previously (Dhingra *et al.*, 2000; Haverkamp & Wässle, 2000) both G α o and PKC α are present both in the dendritic tips and throughout the cell body, whereas G β 5 staining is strikingly concentrated in the dendritic tips, where its signal coincides with PKC α in rod bipolar cells and with G α o in both rod and cone ON-bipolar cells (Fig. 2).

In contrast, G β 5 staining did not coincide with staining for the presynaptic marker ribeye, a synaptic ribbon protein (Fig. 3A), nor did it coincide with staining for calbindin, a marker for the post-synaptic processes of horizontal cells (Fig. 3B), although it was adjacent to both. Thus, all detectable G β 5 staining in the OPL seems to be confined to the post-synaptic bipolar cells. This conclusion is further supported by strong co-localization of G β 5 and mGluR6 (Fig. 3C). To quantify this localization, we averaged twenty 50 $\times$ 50-pixel images centered on G β 5 puncta and determined the intensity distribution profile of each marker (Fig. 3D and E). The advantage of this technique is that the centers of intensity distribution profiles can be located with much greater precision than the nominal resolution of the image, given by the width of the Airy disk. We found that the peak G β 5 signal in immunoreactive puncta was clearly separated from the peak of the ribeye (ribbon) staining by 540 nm, and somewhat closer (340 nm) but still distinct from the peak of calbindin staining (horizontal cell processes). In contrast, the peaks of mGluR6 and G β 5 staining are within one pixel (125 nm) of one another.

RGS7 and RGS11 complexes are present in the OPL

Endogenous G β 5 typically exists in a tightly bound complex with a GGL domain containing RGS protein; for example, in outer segments G β 5L is bound to RGS9. The presence of G β 5 in the OPL suggests that an R7-RGS protein may also be localized in the OPL in a complex with G β 5. We compared the distribution of RGS7, 9 and 11 in mouse retina by immunofluorescent confocal microscopy (Fig. 4A). RGS6 has been shown previously to be confined to the inner retina (Song *et al.*, 2007). As reported (Zhang *et al.*, 2003), strong RGS9 immunoreactivity was detected in the photoreceptor outer segments, with little staining elsewhere. In contrast, both the RGS7 and the RGS11 antibodies intensely labeled the OPL, with faint RGS7 staining also occurring over the outer segments (Fig. 4A). For both RGS7 and RGS11, bright puncta were labeled throughout the OPL associated with rod and cone terminals, similar to that observed for G β 5. The specificity of the antisera against RGS7, 9 and 11 was demonstrated with Western blots of mouse retinal extract in which all three antisera labeled bands at the predicted molecular weight (Fig. 4B).

Immunoprecipitation experiments (Fig. 4C) confirmed that both RGS7 and RGS11 are tightly associated with G β 5 in retinal extracts. Mouse retinal extract was passed over columns coupled with either G β 5 antiserum or preimmune serum. After washing and eluting, the bound proteins were subjected to Western blotting with the G β 5, RGS7 and RGS11 antisera. Total retinal extract was included on the gel as a positive control. All three antisera detected single bands at the predicted molecular weight in the G β 5 immunoprecipitation samples, as well as in the total retinal extract, but not in the preimmune samples, indicating a specific association of RGS7 and RGS11 with G β 5 in the retina.

Co-localization of G β 5–RGS7 and G β 5–RGS11 with mGluR6

Similar to G β 5, double labeling for either RGS7 or RGS11 and mGluR6 revealed extensive overlap of the RGS7 and RGS11 puncta with mGluR6, suggestive of localization of RGS7 and RGS11 to ON-bipolar cell dendrites (Fig. 5A).

To confirm the localization of the complexes to ON-bipolar cell dendrites, RGS11 immunofluorescence was assessed in acutely dissociated rod bipolar cells (Fig. 5B). Rod bipolar cells, identified by PKC α immunofluorescence, were found to contain RGS11 immunofluorescence in their dendrites, confirming the post-synaptic localization of the RGS11 complex to ON-bipolar cells. Similar results were obtained with the G β 5 antibody (not shown).

The strict co-localization of G β 5–RGS7 and G β 5–RGS11 with mGluR6 in the ON-bipolar cell dendrites suggests that these proteins may be localized by a common mechanism, or may depend on one another for localization. We examined this possibility by comparing the distribution of RGS7, RGS11 and G β 5 in wild-type and *nob4* (mGluR6-deficient) mouse retina sections. The *nob4* mouse contains a chemically induced point mutation in the gene encoding mGluR6 (*Grm6*), resulting, immunohistochemically, in no detectable mGluR6 protein in the retina, and physiologically, in an absence of ganglion cell ON-responses (Pinto *et al.*, 2007). Immunohistochemical comparison of ON-bipolar cell-associated proteins between wild-type and *nob4* retinas showed no marked differences in the distributions of PKC α (Pinto *et al.*, 2007), Gao or nyctalopin (data not shown; Morgans *et al.*, 2006). RGS11 (Fig. 6), on the other hand, showed a striking shift in its staining pattern between the wild-type and the *nob4* retinas. A similar alteration in the staining pattern was observed for RGS7 and G β 5 (data not shown). In the *nob4* retina, punctate staining associated with rod terminals was lost from the OPL. Staining associated with cone terminals persisted in the *nob4* OPL, but the intensity and punctate appearance of the staining was diminished. In the *nob4* retina, all three proteins appeared more diffusely distributed throughout the ON-bipolar cells, as staining was detectable in bipolar cell bodies and in the ON-sublamina of the IPL. These data suggest that mGluR6 is required for restricting the G β 5–RGS7 and G β 5–RGS11 complexes to the ON-bipolar cell dendrites.

Discussion

In ON-bipolar cells, activation of mGluR6 by glutamate leads to the closure of a non-selective cation channel and hyperpolarization of the cell. This response depends on the presence of the heterotrimeric Gprotein, Go, specifically the Gao₁ splice variant of the Gao subunit (Dhingra *et al.*, 2002), but other components of the pathway have not yet been identified. Data presented here suggest that the G β 5–RGS7 and G β 5–RGS11 complexes may also be part of the mGluR6 pathway. RGS7 and RGS11 can be co-immunoprecipitated with G β 5 from retinal extracts, and G β 5, RGS7 and RGS11 all co-localize with mGluR6 to the tips of ON-bipolar cell dendrites. In the *nob4* mouse, which lacks mGluR6, the G β 5 complexes are mislocalized, appearing more diffusely distributed through the ON-bipolar cells, suggesting a dependence on mGluR6 for the dendritic localization in the wild-type animal.

Although the downstream targets of Gao in the retina are not known, its broad distribution throughout ON-bipolar cells suggests that it is likely to act in diverse signaling pathways in these cells, and may interact with distinct modulators and effectors in different subcellular compartments. Gyl3, first identified as the γ subunit of gustducin, a transducin-like G protein expressed by taste receptor cells (Huang *et al.*, 1999), has been proposed to interact with Gao in ON-bipolar cells. Gyl3 antibodies selectively label ON-bipolar cells in the retina, and similar to Gao, the staining fills the entire cell from the dendrites to the synaptic terminals (Huang *et al.*, 2003). Likewise, the Ga guanine nucleotide dissociation inhibitor, L7 (pcp-2), fills the entirety of retinal rod bipolar cells (Berrebi *et al.*, 1991). Another potential Gao modulator is

the GAP RET-RGS1 (an isoform of RGS20; Faurobert & Hurley, 1997), which is widely distributed throughout the retina including in photoreceptors, bipolar cells and ganglion cells (Dhingra *et al.*, 2004). It can be co-immunoprecipitated from retinal extracts with Gao (Dhingra *et al.*, 2004), suggesting that it may regulate Gao function in one or more of these locations.

In contrast to the above-mentioned Gao modulator/ effectors, the G β 5-RGS7 and G β 5-RGS11 complexes are the only Gao-interacting proteins identified to date that are confined to the dendritic tips of the ON-bipolar cells, and not broadly distributed through the cell. This restricted localization appears to depend on mGluR6, as it is lost in the *nob4* mouse (Fig. 6). The restricted localization of G β 5-RGS7 and G β 5-RGS11 would serve to regulate Gao activity locally, in the vicinity of mGluR6. The precise co-localization with mGluR6 argues that both RGS7-G β 5 and G β 5-RGS11 may function specifically in the mGluR6 signal transduction pathway.

Such a role for G β 5-RGS7 and/or G β 5-RGS11 complexes in the mGluR6 signal transduction pathway of ON-bipolar cell dendrites would be reminiscent of the role of the G β 5L-RGS9 complex of photoreceptor outer segments, where it deactivates the G α subunit of transducin by stimulating GTP hydrolysis. In outer segments, this GTPase accelerating activity is critical in setting the inactivation kinetics of phototransduction (Krispel *et al.*, 2006). Similar to the action of G β 5-RGS9-1 on G α_T , G β 5-RGS7 and G β 5-RGS11 complexes have been shown to accelerate GTP hydrolysis by Gao in heterologous expression systems (Snow *et al.*, 1999; Lan *et al.*, 2000; Hooks *et al.*, 2003). If G β 5-RGS7 and G β 5-RGS11 act as GTPase accelerators in ON-bipolar cells, they may be responsible for the rapid deactivation of the ON-bipolar cell response to glutamate, allowing a prompt light response. Spontaneous GTP hydrolysis by Gao is too slow to account for the observed kinetics of the ON-bipolar cell light response, which reaches its peak amplitude within 100 ms (Berntson & Taylor, 2000). A working model would be that upon illumination and a concomitant drop in glutamate release from photoreceptors, mGluR6 ceases to activate Gao, and G β 5-RGS7 and G β 5-RGS11 located in the vicinity of mGluR6 rapidly deactivate Gao-GTP, so that the pool of Gao-GTP declines with subsecond kinetics, and the cation channels open. Recently, Rao & Chen (2006) showed that G β 5^{-/-} mice completely lack the b-wave of the electroretinogram, which reflects the ON-bipolar cell response. These data suggest that the GTPase accelerating function of RGS7-G β 5 and/or RGS11-G β 5 is essential for ON-bipolar cell responses.

The presence of both G β 5-RGS7 and G β 5-RGS11 in the ON-bipolar cell dendrites raises the question of whether they are redundant complexes or whether they serve different functions in the ON-bipolar cell pathway. While both G β 5-RGS7 and G β 5-RGS11 have been shown to act as GAPs on Gao, G β 5-RGS11 has been shown to have twice the GAP activity of G β 5-RGS7 (Hooks *et al.*, 2003). The same study revealed that co-expression of G β 5-RGS7 with G β 5-RGS11 inhibited the GAP activity of G β 5-RGS11 to levels closer to G β 5-RGS7, suggesting interactions between the two complexes. The GAP activities of G β 5-RGS7 and G β 5-RGS11 are likely to be differentially regulated. RGS7, for example, has been shown to interact with an array of signaling molecules including R7BP (Martemyanov *et al.*, 2005), 14-3-3, PKC α , phosphatidylinositol-3, 4, 5-triphosphate (PIP3) and calmodulin (Benzing *et al.*, 2002; Liu *et al.*, 2005).

The molecular identity of the signaling cascade in ON-bipolar cells is a long-standing mystery in retinal physiology. The immunohisto-chemical evidence presented in this study suggests that G β 5-RGS7 and G β 5-RGS11 are likely to be key players in the cascade, and important determinants of the kinetics of the ON-bipolar cell light response.

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Abbreviations

BPC, bipolar cell; GAP, GTPase-accelerating protein; IPL, inner plexiform layer; OPL, outer plexiform layer; RGS, regulator of G-protein signaling.

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FIG. 1.

Gβ5 is present in the outer plexiform layer of the retina. (A) Immunofluorescent labeling of a mouse retinal section for Gβ5. All staining is blocked by pre-incubation of the antibody with the peptide immunogen (+ pep). (B) High-power view of Gβ5 labeling in the OPL. Labeling is associated with both rod and cone (arrows) terminals. (C) Fixation dependence of Gβ5 labeling demonstrated with retinas fixed for either 15 min or 18 h in 4% paraformaldehyde. (D) Western blot of mouse retina extract with the Gβ5 antiserum. Scale bars represent 50 μm in A, 5 μm in B, and 25 μm in C. Abbreviations: OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

FIG. 2.

Gβ5 is located in the tips of ON-bipolar cell dendrites. Mouse retina sections were double labeled for (A) Gβ5 (red) and Gαo (green), and (B) Gβ5 (red) and the rod bipolar cell marker PKCα (green). Scale bar in A represents 10 μm and also applies to B.

FIG. 3.

Gβ5 is localized post-synaptically in ON-bipolar cell dendrites. (A) Gβ5 (red) and the synaptic ribbon protein ribeye (green), (B) Gβ5 (red) and the horizontal cell marker calbindin (green), or (C) Gβ5 (red) and mGluR6 (green). Areas of co-localization appear yellow. (D) Averaged images centered on the Gβ5 puncta in A–C. (E) Plots of the corresponding channel intensity profiles. The scale bars represent 5 μm in A, and 10 μm in B and C.

FIG. 4.

RGS7 and RGS11 are localized to the OPL and can be immunoprecipitated from retinal extracts with Gβ5. (A) Mouse retina sections were labeled by immunofluorescence for RGS7, RGS9 and RGS11. The left panel shows a DIC image of a mouse retina section with the layers indicated. The scale bar represents 20 μm, and applies to all panels. (B) Immunoblots of mouse retinal extract with antisera against RGS7, 9 and 11. (C) Mouse retinal extract was immunoprecipitated with anti-Gβ5 (+) or preimmune serum (-), and then Western blotted for Gβ5, RGS7 and RGS11. Included on the blots were lanes containing retinal extract (ext). Abbreviations: OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer.

FIG. 5. RGS7 and RGS11 co-localize with mGluR6 in the OPL. (A) Mouse retina sections were double labeled for mGluR6 (green) and either RGS7 or RGS11 (red). Areas of co-localization appear yellow. The scale bar represents 10 μ m and applies to all panels. (B) Acutely dissociated mouse rod bipolar cells were double labeled for RGS11 (green) and PKC α (red).

FIG. 6. RGS11 is mislocalized in the *nob4* retina. Immunofluorescent localization of mGluR6 (top panels) and RGS11 (bottom panels) in wild-type (left) and *nob4* (right) retinas. Abbreviations: ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer. The scale bar represents 20 μm and applies to all panels.