The C-terminal Tail of the Metabotropic Glutamate Receptor Subtype 7 Is Necessary but Not Sufficient for Cell Surface Delivery and Polarized Targeting in Neurons and Epithelia*

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Complex neuronal functions rely upon the precise sorting, targeting, and restriction of receptors to specific synaptic microdomains. Little is known, however, of the molecular signals responsible for mediating these selective distributions. Here we report that metabotropic glutamate receptor subtype 7a (mGluR7a) is polarized at the basolateral surface when expressed in Madin-Darby canine kidney (MDCK) epithelial cells but is not polarized when expressed in cultured hippocampal neurons. Truncation of the mGluR7 cytoplasmic tail produces a protein that is restricted to a perinuclear intracellular compartment in both neurons and MDCK cells, where this protein colocalizes with a trans-Golgi network antigen. The mGluR7 cytoplasmic domain appended to the transmembrane portion of the vesicular stomatitis virus G protein and the ectodomain of human placental alkaline phosphatase is distributed over the entire cell surface in cultured neurons. When expressed in MDCK cells, this construct remains in an intracellular compartment distinct from endosomes or lysosomes. Thus, the cytoplasmic tail domain of mGluR7 is necessary but not sufficient for polarized targeting in MDCK monolayers, whereas in neurons the cytoplasmic tail is sufficient for cell surface expression but not polarization. Additional mechanisms are likely required to mediate mGluR7 neuronal polarization and synaptic clustering.

Specialized domains at the neuronal cell surface orchestrate complex synaptic functions. Accurate sorting, trafficking, and restriction of proteins to these microdomains ensures the precise distribution of synaptic proteins for proper neuronal function. Proteins destined for secretion or incorporation into the plasma membrane transit to the cell surface through a series of subcellular compartments. Synthesis occurs in the soma at the rough endoplasmic reticulum (RER), and postsynaptic processing continues in the Golgi complex, with sorting of proteins into specific transport vesicles occurring in the TGN (1, 2). Information contained within targeted proteins function as sorting signals to specify their ultimate destination within the cell (3, 4). Although progress is being made in the categorization of sorting signals, their diversity and complexity, as well as the underlying mechanisms through which cells carry out polarized targeting, remain unresolved (1).

Neurotransmitter receptors are among the complex array of proteins at synaptic microdomains that participate in the generation and propagation of neuronal activity. Glutamate serves as the principle excitatory amino acid neurotransmitter at central nervous system synapses. Activity at glutaminergic synapses is mediated by both ionotropic and metabotropic glutamate receptors (5). Metabotropic glutamate receptors couple with heterotrimeric G-proteins to regulate cell excitability and synaptic transmission (6, 7) and have been implicated in synaptic plasticity (8). Currently eight distinct mGluR subtypes have been recognized (6). These have been observed to exhibit subtype- and region-specific differences in distribution along the neuronal cell surface in vivo. The mGluR7 subtype, in particular, highlights the complexity of protein targeting. mGluR7 receptors have been reported to be targeted to postsynaptic sites in the olfactory bulb (9) and locus coerules (10, 11), to axons in the hippocampus (12, 13), to both pre- and postsynaptic sites in the retina (14), and to both axons and dendrites of cultured hippocampal neurons (15). In the hippocampal formation additional complexity is evident, because the distribution of mGluR7 is apparently dependent upon the nature of the postsynaptic target neuron. Within individual axons, mGluR7 is found to be present only in distinct subpopulations of presynaptic terminals (16). The subtype- and region-specific placement of mGluR7 at distinct membrane domains requires the existence of mechanisms whereby sorting information is interpreted, so that receptors can be distributed precisely. mGluR7 is a member of the larger seven transmembrane or G-protein-coupled receptor family of receptors. Little is known about the cellular mechanisms that govern the intracellular trafficking of the individual members of this critically important protein family.

Because of the technical difficulties inherent in studying sorting and trafficking in neurons, cultured cell lines have been utilized as a means both to study sorting in a polarized cell...
site was introduced at the Bluescript polynucleotide NotI site by insertion of an oligonucleotide linker. This XhoI site was used along with the pBluescript polynucleotide Clal restriction site and the AsclII restriction site within mGluR7a for subcloning and fusion protein construction. Fusion PCR was used to construct the GFP-mGluR7 fusion protein. PCR was used to generate three products from which the final fusion products were made by use of the same primers. The first PCR product was generated from the mGluR7 wt cDNA using a 5′-primer complimentary to and including the pBluescript polylinker XbaI site and the AsclII restriction site of the intended GFP incorporation (10 amino acids following the signal sequence) and the first seven codons of the GFP sequence. The GFP sequence was generated in a similar manner from pEGFP-C1 (CLONTECH, Palo Alto, CA) using primers that included both the mGluR7 sequence before the intended insertion site and the sequence on either end of the GFP encoding sequence. The downstream mGluR7 product was generated with a 5′-primer including both the first five codons of GFP and the initial six codons following the final transmembrane domain and an XhoI restriction site. The resulting fragment was digested at the unique Clal and AsclII restriction sites for replacement into the original cDNA plasmid of mGluR7. The GFP-mGluR7-tail deletion construct was generated by PCR with a 5′-primer at the interface of the GFP and mGluR7 encoding sequence. The downstream mGluR7 product was generated with a 5′-primer containing the polylinker (noncell surface) perinuclear distribution was observed for the GFP-mGluR7-tail-minus receptor construct as determined by either anti-GFP antibody staining (panel d) or via the intrinsic GFP fluorescence signal. We report that mGluR7 achieves a polarized distribution at the basolateral membrane when expressed in both cell types. We report that mGluR7 is necessary but not sufficient for this polarized targeting in MDCK cells. In neurons however, exogenously expressed mGluR7 does not achieve a polarized distribution. The cytoplasmic tail is not sufficient for polarization, but without it the protein does not achieve a cell surface distribution.

**EXPERIMENTAL PROCEDURES**

**Construction of Fusion Proteins**—Cloned mGluR7a cDNA in the pBluescript plasmid was a kind gift from S. Nakanishi (20). An XhoI product was then used in a similar manner with the third PCR product and the first seven codons of the GFP sequence. The GFP sequence was generated from the mGluR7 wt cDNA using a 5′-primer complimentary to and including the pBluescript polylinker XbaI site and the AsclII restriction site of the intended GFP incorporation (10 amino acids following the signal sequence) and the first seven codons of the GFP sequence. The GFP sequence was generated in a similar manner from pEGFP-C1 (CLONTECH, Palo Alto, CA) using primers that included both the mGluR7 sequence before the intended insertion site and the sequence on either end of the GFP encoding sequence. The downstream mGluR7 product was generated with a 5′-primer including both the first five codons of GFP and the initial six codons following the final transmembrane domain and an XhoI restriction site. The resulting fragment was digested at the unique Clai and AsclII restriction sites for replacement into the original cDNA plasmid of mGluR7. The GFP-mGluR7-tail deletion construct was generated by PCR with a 5′-primer at the interface of the GFP and mGluR7 encoding sequence. The downstream mGluR7 product was generated with a 5′-primer containing the polylinker (noncell surface) perinuclear distribution was observed for the GFP-mGluR7-tail-minus receptor construct as determined by either anti-GFP antibody staining (panel d) or via the intrinsic GFP fluorescence signal. We report that mGluR7 achieves a polarized distribution at the basolateral membrane when expressed in both cell types. We report that mGluR7 is necessary but not sufficient for this polarized targeting in MDCK cells. In neurons however, exogenously expressed mGluR7 does not achieve a polarized distribution. The cytoplasmic tail is not sufficient for polarization, but without it the protein does not achieve a cell surface distribution.

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**FIG. 1.** Metabotropic glutamate receptor constructs. The predicted membrane topologies and relevant sequence domains are illustrated for each mGluR7 cDNA construct. mGluR7 wt contains the entire wild-type receptor. The GFP-mGluR7 fusion protein (GFP-mGluR7) has an extracellular GFP incorporated inframe at a position 10-amino acids distal to the signal sequence. PLAP-mGluR7 tail has the mGluR7 cytoplasmic tail incorporated inframe with a single spanning membrane protein containing the extracellular placental alkaline phosphatase domain and the VSVG transmembrane domain. GFP-mGluR7 tail minus represents the GFP fusion receptor protein with its cytoplasmic tail deleted.

**FIG. 2.** Cellular distribution of mGluR7 receptor constructs transiently expressed in COS-1 cells determined by immunofluorescence. COS-1 cells were transiently transfected with mGluR7 receptor cDNAs constructed in the pcB6 mammalian expression vector, and exogenous protein localization was determined by fluorescence microscopy. Cell surface distributions were demonstrated for three of the mGluR7 constructs: mGluR7 wt as determined with an anti-mGluR7 antibody (panel a), GFP-mGluR7 as determined by intrinsic GFP fluorescence signal (panel b), and PLAP-mGluR7-tail as determined with an anti-PLAP antibody (panel c). However, an intracellular (noncell surface) perinuclear distribution was observed for the GFP-mGluR7-tail-minus receptor construct as determined by either anti-GFP antibody staining (panel d) or via the intrinsic GFP fluorescence signal.
Fig. 3. Cellular distribution of mGluR7-wt and GFP-mGluR7 stably expressed in polarized MDCK cells determined by immunofluorescence. MDCK cells were stably transfected with each mGluR7 construct in the pCB6 mammalian expression vector. Confocal en face (a, c, d, f, g, i, j, l) and confocal xz cross-section (b, e, h, k) immunofluorescence images of MDCK cells were obtained. a–c illustrate an intracellular distribution of mGluR7 wt as determined by staining with anti-mGluR7-wt. c, the intracellular distribution of mGluR7 is highlighted (arrowheads). The GFP:mGluR7 receptor construct displays a mainly basolateral cell surface distribution (d–f) in MDCK cells as determined by native GFP fluorescence. f, minimal intracellular mGluR7 is observed (arrowheads). In contrast, however, immunofluorescence labeling of GFP:mGluR7 with the cytoplasmic tail-directed anti-mGluR7-wt antibody (g–i) shows a primarily intracellular distribution (i, arrowheads) as observed in c. Immunofluorescence labeling of GFP:mGluR7 with anti-GFP (j–l) illustrates both extracellular and additional intracellular labeling as compared with (d–f). The intracellular mGluR7 is highlighted (l, arrowheads).

Fig. 4. Cellular distribution of PLAP-mGluR7 and GFP-mGluR7-tail-minus receptor constructs in polarized MDCK cells as determined by immunofluorescence. MDCK cells were stably transfected with PLAP-mGluR7-tail and GFP-mGluR7-tail-minus in the pCB6 mammalian expression vector. Confocal en face immunofluorescence images of MDCK cells are presented. In a and b, immunofluorescence of PLAP:mGluR7-tail shows the construct to be contained in a punctate intracellular compartment as determined by staining with anti-PLAP. In c and d, immunofluorescence of GFP-mGluR7-tail-minus illustrates the construct to be contained in a different intracellular compartment, which shows a perinuclear distribution similar to that seen in Fig. 2.

HindIII restriction site, and a 3'-primer to incorporate an XbaI restriction site following the stop codon. The resulting fragment was digested at the XbaI and HindIII restriction sites for replacement into the cDNA plasmid of PLAP-VSVG. All constructs were sequenced through the respective ligation points. The constructs were then subcloned into the mammalian expression vector pCB6 (kindly provided by M. Roth, University of Texas, Southwestern) between the ClnI and XbaI sites prior to transfection.

Cell Culture and Transfection—COS-1 cells were transiently transfected using DEAE-dextran (22). Cells (2 x 10⁶ per 35-mm dish) were plated on glass coverslips the night before transfection. Cells were washed in Tris-buffered saline and exposed to 5 µg of DNA, and 0.5 mg/ml DEAE-dextran (Amersham Pharmacia Biotech) was added for 40 min at 37 °C. The solution was discarded, and the cells were incubated in 1 ml of DMEM containing 10% fetal bovine serum and 0.1 mM chloroquine for 3 h at 37 °C. The cells were washed in Tris-buffered saline and incubated in complete DMEM for 48–72 h prior to immunofluorescence immunohistochemistry. Subconfluent MDCK cells were prepared as previously described (22) and transfected by the PerFect lipid method (Invitrogen, San Diego, CA), with fusion constructs that had been subcloned into the pCB6 vector. This vector carries resistance to the antibiotic G418 (Life Technologies, Inc.). After selection in 1.8 mg/ml G418, clones were screened for expression by immunofluorescence, Western blotting, or phosphatase assay (BluePhos Microwell, Kirkegaard & Perry, Gaithersburg, MD).

Primary hippocampal cultures were prepared as described (19). Cultured neurons were transfected at stage 5, a stage when synapses have been formed and endogenous synaptic receptors have polarized and localized to the synapses. Cultured hippocampal neurons were transfected at 7 days in vitro (DIV) by the LipofectAMINE 2000 method (Life Technologies). In brief, 1 µg of DNA was diluted into 50 µl of serum-free medium and incubated at room temperature for 5 min. Then, 3 µl of LipofectAMINE 2000 reagent was diluted into 50 µl of serum-free medium in a second tube. The diluted DNA and the diluted LipofectAMINE 2000 reagent were combined, mixed, and incubated for 15 min at room temperature. Neurons were transfected to a new 6-well plate that contained 1 ml of the neuronal maintenance medium from the hippocampal culture. The DNA-LipofectAMINE 2000 reagent complexes were mixed into the medium bathing the cells and incubated at 37 °C with 5% CO₂ for 2 h. The coverslips were then placed back into the original 6-well plate and incubated for 2 days.

Immunofluorescent Microscopy—Immunofluorescent microscopy was performed as described (4) on stably expressing cell lines. Briefly, cells were grown to confluence on Transwell porous cell culture inserts (Corning Costar Corp., Cambridge, MA) or, for screening clones, on 8-well Lab-Tek slides (Nalge Nunc International, Naperville, IL) and fixed in −20 °C methanol for 7 min at room temperature. Cells were then permeabilized in a phosphate-buffered saline-based wash buffer containing 0.3% Triton X-100 and 0.1% bovine serum albumin for 15 min. Nonspecific binding of antibody was blocked by incubating the cells in goat serum dilution buffer (16% filtered goat serum, 0.3% Triton X-100, 20 mM NaPi, pH 7.4, 0.9% NaCl) at room temperature for 1 h. Chimeras were detected either by native GFP fluorescence, anti-mGluR7 wt (kindly provided by J. Conn, Ref. 12), anti-GFP (CLONTECH Laboratories, Inc.), or anti-PLAP (Fitzgerald Inc.) (1:50). Secondary goat anti-mouse or anti-rabbit antibodies (1:200) were conjugated to either rhodamine or fluorescein (Sigma). All antibody incubations took place in goat serum dilution buffer for 1 h at room
either the apical (transient) biotinylation analysis did not detect the PLAP (lanes 1, 2) or the basolateral (lanes 3, 4) cell surface. Lane 5 illustrates the absence of the construct at the surface of untransfected MDCK cells. The GFP-mGluR7-tail-minus construct was not detected at either the apical (lanes 6, 7) or the basolateral (lanes 8, 9) cell surface. Lane 1, consistent with its intracellular distribution (Fig. 4), cell surface biotinylation analysis did not detect the PLAP-mGluR7-tail construct at either the apical (lanes 2, 3) or basolateral (lanes 4, 5) cell surfaces. To ensure that the transfected MDCK cells express the PLAP-mGluR7-tail protein, Western blot analysis was performed on total cell lysates, revealing the presence of a protein of ~65 kDa corresponding to the construct.

temperature. Between primary and secondary antibody incubations, the cells were subjected to three 5-min washes in the phosphate-buffered saline-based wash buffer. After incubation with the secondary antibody, cells were washed in phosphate-buffered saline three times for 5 min each and finally in 10 mM NaPi for 10 min before being mounted on coverslips with Vectashield (Vector Laboratories, Burlingame, CA). Confocal sections were taken using a Zeiss LSM 410 laser scanning confocal microscope. Images are the product of 8-fold line averaging. Xz cross sections were generated with a 0.2 micron motor step. Contrast and brightness were set so that all pixels were in the linear range. Immunofluorescence staining of neurons was performed as described (19). Immunofluorescent images were captured with IP Labs on a Zeiss Axiohot epifluorescence photomicroscope. Fluorescent dextran uptake was performed as described (23), cells were incubated for various times with DMEM + containing 1 mg/ml rhodamine-dextran (lysine fixable) (Molecular Probes) Ms = 10,000 in both upper and lower Transwell filter chambers.

Cell Surface Biotinylation—Cells were grown on 24-mm Transwell inserts (0.4-µm pore, Costar) for 1 week, and the medium was replaced with complete MEM (without Geneticin) before the experiment. Steady-state biotinylation of either apical or basolateral proteins was carried out separately at pH 9.0 as described (22). Cells were incubated with N-hydroxysuccinimide (NHS)-biotin for 2× 20 min. Following biotinylation, filters were excised from the cups with a razor blade, and the attached monolayers were lysed in 1% Triton X-100 in 150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.5. The biotinylated proteins (either apical or basolateral cell surface proteins) were recovered from cell lysates by incubation with 100 µl of packed immobilized streptavidin-agarose beads (Pierce). Bound proteins were eluted from the beads in Laemmli sample buffer, analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting as described previously (22). The blot was incubated with anti-GFP or anti-PLAP (1:200) primary antibodies followed by horse radish peroxidase-conjugated secondary antibodies (Sigma) and developed with enhanced chemiluminescence reagent (Amersham Pharmacia Biotech).

RESULTS

Metabotropic Glutamate Receptor Constructs: Substitution, Deletion, and GFP Fusion Protein Constructs—mGluR7 is a 7-transmembrane domain receptor with a large extracellular domain and a 60-amino acid cytoplasmic tail. The construct designated R7 wt (Fig. 1, mGluR7 wt) was investigated by use of a specific antiserum (11) directed against the C-terminal cytoplasmic tail of mGluR7. For subsequent investigations we chose to epitope-tag the receptor by creating GFP-mGluR7 fusion proteins. The GFP-R7 fusion protein construct has GFP incorporated in frame at a position 10 amino acids distal to the predicted end of the final transmembrane domain creating the GFP-R7-tail-minus construct (Fig. 1, GFP-mGluR7 tail minus). To investigate whether the cytoplasmic tail of mGluR7 may function autonomously to direct localization, a fusion protein PLAP-R7-tail (Fig. 1, PLAP-mGluR7 tail) was generated with the mGluR7 cytoplasmic tail incorporated in-frame with a construct containing the VSVG transmembrane domain and placental alkaline phosphatase as the extracellular domain.

R7 wt and GFP-R7 Fusion Protein Distribution in Polarized and Nonpolarized Cells—Constructs were transiently expressed in COS-1 cells. Both R7 wt (Fig. 2a) and GFP-R7 (Fig. 2b) attain a cell surface distribution in COS-1 cells. Stably transfected MDCK cells also expressed mGluR7 at the plasma membrane. MDCK cell lines expressing mGluR7 were grown to confluence and examined by indirect immunofluorescence confocal microscopy (Figs. 3 and 4) using the anti-mGluR7 antiserum for R7 wt and either native GFP fluorescence or anti-GFP for GFP-R7. GFP-R7 displayed a polarized steady-state distribution at the basolateral surface of MDCK epithelial cells (Fig. 3, d, e, and f). To assess biochemically the polarized distributions of the mGluR7 proteins, we employed the cell surface biotinylation technique to differentially isolate MDCK apical and basolateral proteins. Steady-state cell surface biotinylation supported the immunofluorescent observation, by confirming GFP-R7 distribution at the basolateral surface (Fig. 5A).

To examine how neurons might sort and target GFP-R7, we determined whether the construct is appropriately targeted and polarized at steady state in cultured neurons. Use of the GFP fusion construct allowed detection of only exogenously expressed mGluR7. Whereas the majority of the immunofluorescence signal for GFP-R7 was intracellular or perinuclear, GFP-R7 was found at the cell surface throughout both axons and dendrites and with no obvious clustering. Exogenously expressed mGluR7 did not achieve a polarized distribution in cultured hippocampal neurons and was found at apparently equal intensity at the cell surfaces of both axons and dendrites (see Fig. 8, a and b).

Sorting of the Cytoplasmic Tail of mGluR7 in MDCK Cells—Comparison of R7 wt and GFP-R7 fusion protein distributions suggests that the C-terminal tails of these proteins participate in a macromolecular complex. In MDCK cells stably expressing R7 wt, the protein distribution appears to be intracellular (Fig. 3, a–c) when immunolabeling is performed using an antiserum directed against the final 18 amino acids of the mGluR7 carboxyl terminus. Importantly, when examined using the same C-terminal-directed antiserum, the distribution of GFP-R7 also appears to be intracellular (Fig. 3, g–i). In con-
trast, observation of the GFP intrinsic GFP signal in the same field (Fig. 3, d–f), together with cell surface biotinylation (see Fig. 5A), clearly illustrates a cell surface distribution (at the basolateral plasmalemma, Fig. 3, d–f). These data suggest that in MDCK cells, the C-terminal portion of cell surface mGluR7 is interacting with a protein or proteins that interfere sterically with access of the C-terminal antibody to its epitope. According to this hypothesis, the C termini of mGluR7 intracellular populations do not participate in these interactions, thus permitting their detection in immunohistochemical experiments employing the C-terminal antibody to its epitope. According to this hypothesis, the C termini of mGluR7 intracellular populations do not participate in these interactions, thus permitting their detection in immunohistochemical experiments employing the C-terminal antibody to its epitope. Observation of the GFP-R7 cell line with anti-GFP is consistent with this conclusion, revealing intracellular labeling (Fig. 3, j–i) in addition to the population detected at the cell surface. It would appear, therefore, that incorporation of mGluR7 into the basolateral plasma membrane of MDCK cells is most likely associated with the formation of protein–protein interactions involving its C-terminal tail.

Importance of the mGluR7 Cytoplasmic Tail for Sorting—Because the mGluR7 C-terminal tail appears to participate in protein–protein interactions at the MDCK cell basolateral membrane, we wondered whether the tail embodies information needed to establish protein localization. To test this possibility, we examined the distributions of the GFP-R7-tail-minus and the PLAP-R7-tail constructs.

Following transient expression in COS-1 cells, GFP-R7-tail-minus localized to perinuclear intracellular compartments whose morphology and distribution are reminiscent of the Golgi complex (Fig. 2). When these constructs are expressed in MDCK cells, the GFP-R7-tail-minus construct recapitulated its COS cell distribution and was found in a perinuclear intracellular compartment (Fig. 4, c and d). To further identify the intracellular compartment in which the GFP-R7-tail-minus accumulates; we performed double labeling with an antibody specific to a Lupus auto-antigen expressed in the TGN (provided by A. Gonzalez; Ref. 24). In both COS-1 cells (Fig. 6, a–c) and in MDCK cells (Fig. 6, d–f and g–i) we found that GFP-R7-tail-minus colocalizes with the TGN marker. In addition, the Golgi population of this tail-minus construct does not seem to be able to cycle to the cell surface and return to the TGN via endosomes, as has been noted for TGN38 and furin. In the case of furin and TGN38, return to the TGN requires endosomal acidification and is inhibited by agents that raise endosomal pH (25). Incubation with chloroquine (100 micromolar, 4 h) did not alter GFP-R7-tail-minus distribution in MDCK cells (data not shown). The perinuclear Golgi-like distribution
did not change and did not disperse to a characteristic punctate endosomal distribution during treatment. Thus, the tail-minus construct appears to be a fairly stable resident of the TGN that does not traffic from the cell surface via endosomes.

When transiently expressed in COS-1 cells, the PLAP-R7-tail construct attained a cell surface distribution (Fig. 2c). However, the PLAP-R7-tail construct did not attain a cell surface distribution in MDCK cells. Instead it was concentrated in a dispersed, punctate intracellular distribution (Fig. 4, a and b). This compartment is not labeled by antibodies to the lysosomal membrane protein LAMP-1 (Fig. 7, a–c). Subsequent experiments also indicated that it does not become labeled by internalized fluorescein isothiocyanate-dextran (Fig. 7, d–f). It would appear, therefore, that this compartment is neither lysosomal nor endosomal in origin. Steady-state cell surface biotinylation of MDCK monolayers supported immunofluorescent observations, confirming that neither PLAP-R7-tail nor GFP-R7-tail-minus were present at the cell surface (Fig. 5, A and B).

Expression in neurons was performed to test how neurons would distribute the tail-containing and tail-minus constructs at steady state. Immunostaining with anti-PLAP and anti-GFP antibodies enabled detection of only exogenously expressed constructs. In contrast to results in MDCK cells, the immunofluorescence signal for PLAP indicated that PLAP-R7-tail was expressed at the neuronal cell surface throughout both axons and dendrites. It was not distributed in a polarized manner and exhibited no obvious clustering (Fig. 8, e and f). Once again, when exogenously expressed in neurons, GFP-R7-tail-minus was observed in a perinuclear intracellular distribution consistent with a Golgi-like pattern (Fig. 8c), with no observable label at the cell surface (Fig. 8d).

**DISCUSSION**

Neuronal function relies upon the polarized distribution of synaptic proteins. The mGluR7 subtype is a protein distributed with polarity in neurons, being found only in highly selective locations at cell surface synaptic sites. In different brain regions, mGluR7 can be found selectively distributed in either pre- or postsynaptic locations. In particular, mGluR7 distributes to selective subpopulations of axon terminals within individual neurons of the hippocampus. Recently, expression studies have examined the distribution of mGluR7 heterologously expressed in cultured hippocampal neurons (15). Results indicated that full-length mGluR7 was present at the cell surface of both axons and dendrites, suggesting that the exogenously expressed protein did not achieve a polarized distribution. The authors of this study proposed that the C terminus of mGluR7 contains an axonal targeting signal, because it permitted somatodendritic mGluR2 to enter axons.

The molecular nature of the mGluR7 sorting signal remains undefined. We wished therefore, to further examine the signals and mechanisms involved in mGluR7 sorting. Toward this end, we expressed mGluR7 and a variety of receptor constructs in neurons and epithelial cells. These constructs included full-length mGluR7, a GFP fusion construct of full-length mGluR7, mGluR7 without its cytoplasmic tail, and a construct where the cytoplasmic tail of mGluR7 was appended to a single pass transmembrane protein. Consistent with the findings of Stowell and Craig (15), we find that in cultured hippocampal neurons, mGluR7 is expressed in a nonpolarized distribution at the surfaces of both somatodendritic and axonal domains. However in MDCK epithelia, exogenously expressed mGluR7 is polarized at the basolateral cell surface. Further experiments illustrated that the cytoplasmic tail of mGluR7 was necessary for
mGlur7 Targeting; C-terminus Is Necessary but Not Sufficient

Therefore, that the cytoplasmic tail-minus construct seems unable to exit the Golgi. The mechanism of this Golgi retention is not clear. We can state, however, that the protein does not appear to cycle between Golgi and cell surface via endosomes. This cycling behavior is observed for other proteins concentrated in the TGN, including furin and TGN 38. Membrane protein traffic from the TGN to the cell surface has been theorized to involve carbohydrate moieties as either targeting signals (30) or for modulation of the state of protein aggregation (31). In the case of mGlur7-tail-minus, glycosylation sites remain intact. Thus, the cause of retention may be more related to an inability to participate in protein-protein interactions important for stable surface expression. In addition, the cytoplasmic tail of mGlur7 is clearly not sufficient to mediate polarized sorting in epithelia, where the PLAP-mGlur7-tail construct remained within the cell in a cytoplasmic pool distinct from endosomal or lysosomal compartments. In neurons, however, this construct was present at the surface of both axons and dendrites, as was found for exogenously expressed mGlur7, but did not achieve a polarized distribution. Thus, the capacity of mGlur7 to mediate cell surface expression is highly dependent upon the cell type in which they are expressed. The mGlur7 cytoplasmic tail is sufficient for cell surface delivery in neurons, but not in MDCK cells. Importantly, this portion of the protein is not by itself sufficient to specify polarized targeting in either cell type.

It is interesting to note that the cytoplasmic tails of a number of GPCRs interact with PDZ-containing cytoskeletal proteins (32). Cell surface machinery may be vital to polarized sorting (33). Interaction with cytoskeletal elements, particularly PDZ-containing proteins, have been implicated not only in cell surface interactions that retain proteins at specific locations (34), but also at earlier stages of protein targeting at the TGN (35). This is consistent with our observation that the cytoplasmic tail epitope is obscured in MDCK cells, where mGlur7 is distributed in a polarized fashion, but not in COS cells, which lack polarized membrane domains. Physical interaction of putative PDZ-containing cytoskeletal proteins with the C-terminal tail of mGlur7 could explain our inability to observe the construct at the basolateral surface with an antiserum directed to the cytoplasmic tail. In fact, the three final C-terminal residues of mGlur7 may represent a possible hydrophobic class II PDZ interaction motif (36). However, the absence of observable clustering or detergent insolubility for mGlur7 (data not shown) do not support a stable or continuous interaction with the MDCK cytoskeleton, as has been correlated with proper localization of some membrane proteins in MDCK (37, 38).

In summary, the cytoplasmic tail of mGlur7 is clearly necessary for the surface delivery of the receptor but not sufficient to mediate its polarized distribution in epithelia or in neurons. Other domains of the mGlur7 molecule must be involved in contributing to sorting information. The nature of these domains, the signals they encode, and the interactions that interpret them remain to be determined.

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