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ORIGINAL PAPER

Calcium channel γ subunits: a functionally diverse protein family

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Abstract The calcium channel γ subunits comprise an eight-member protein family that share a common topology consisting of four transmembrane domains and intracellular N- and C-termini. Although the first γ subunit was identified as an auxiliary subunit of a voltage-dependent calcium channel, a review of phylogenetic, bioinformatic, and functional studies indicates that they are a functionally diverse protein family. A cluster containing γ_1 and γ_6 conforms to the original description of the protein family as they seem to act primarily as subunits of calcium channels expressed in muscle. Members of a second cluster (γ_2, γ_3, γ_4, γ_8) function as regulators of AMPA receptor localization and function in the brain and are collectively known as TARPs. The function of members of the third cluster (γ_5, γ_7) remains unclear. Our analysis shows that the members of each cluster contain conserved regulatory motifs that help to differentiate the groups. However, the physiological significance of these motifs in many cases remains to be demonstrated.

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
LVA/HVA	low-/high-voltage activated
PDZ	PSD-95/DLG/ZO-1
PKC	protein kinase C
PKA/G	cAMP/cGMP-dependent protein kinase

Introduction

Voltage-dependent calcium channels (VDCCs) are important regulators of calcium influx and consequently help to regulate numerous physiological processes including synaptic transmission, muscle contraction, gene expression, hormone secretion, cell motility, cell division, and development. Structurally, VDCCs are heteromeric proteins composed of a pore-forming α_1 subunit and, potentially, as many as three auxiliary subunits: $\alpha_2\delta$, β, and γ [17]. The α_1 subunits contain the permeation pathway for calcium ions and the voltage sensing regions that control channel activation and gating. Thus, they determine to a great extent the major characteristics of the calcium current carried by the different calcium channel subtypes. The auxiliary subunits $\alpha_2\delta$ and β act, in a sense, as positive regulators of VDCC current. When co-expressed with the α_1 subunit they enhance

trafficking of the channel complex to the surface membrane thus increasing current density [15, 31]. Additionally, $\alpha_2\delta$ and β alter the biophysical properties of calcium channels in ways that enhance activation and/or opening probability [15, 31, 38].

Our current understanding of the cellular processes influenced by the γ subunits suggests a more diverse and complex range of regulatory functions than found with the other calcium channel auxiliary subunits. The γ subunits interact not just with calcium channels but with other proteins as well. In fact, recent evidence suggests that the principle cellular targets of several members of the calcium channel γ subunit family may not be calcium channels at all. The first γ subunit described, γ_1, was isolated biochemically as a component of a calcium channel expressed in skeletal muscle and has been shown to alter calcium current properties in both native myocytes and in cell lines [16, 19, 25, 47]. Historically, as additional members of this protein family were identified by sequence homology to γ_1, it was assumed that their functional roles were also homologous. It now seems clear that this assignment of common function may not have been appropriate. Four of the eight γ subunits (γ_2, γ_3, γ_4, γ_8) are known to act as transmembrane AMPA receptor regulatory proteins (TARPs). Their major cellular function seems to be the regulation of trafficking and gating of AMPA receptors [40, 43]. Thus the assumption that all γ subunit proteins act primarily as regulators of calcium channel function is likely to be incorrect.

Several recent review articles provide excellent summaries of the different aspects of γ subunit function including their roles as regulators of calcium current [1, 4, 18, 29] and as regulators of AMPA receptor localization and function [40, 43]. In this article, we present new bioinformatic analysis and discuss existing structural and experimental evidence that together support multiple function roles for the members of this protein family.

Calcium channel γ subunits are members of the tetraspanin supergroup of membrane proteins

The eight calcium channel γ subunits share a predicted structure that includes four transmembrane domains with intracellular N- and C- termini (Fig. 1). They are members of a large protein superfamily (pfam00822, a subset of the tetraspanin supergroup) that also includes claudins, proteins that are important components of tight junctions in epithelia [53]. The γ subunits share with the claudins a conserved GLW motif of unknown significance in the first extracellular loop.

Phylogenetic analysis suggests that all γ subunits evolved from a single ancestral gene through tandem repeat and chromosome duplication [7, 13]. Based on sequence homology and chromosomal linkage the γ subunits can be divided into three clusters: (γ_1, γ_6), (γ_5, γ_7), and (γ_2, γ_3, γ_4, γ_8) [7, 13]. Interestingly, these clusters also mirror to some extent the tissue distribution of the subunits and their likely cellular functions.

The γ_1 and γ_6 subunits are distinguished from the other γ subunits by their very short C-terminal cytoplasmic regions that lack functional motifs. γ_1 and γ_6 are also unique in that they are predominantly expressed in striated muscle. While the γ_1 subunit seems to be exclusively expressed in skeletal muscle, the γ_6 subunit is expressed in both skeletal and cardiac muscles, and to a lesser extent, in brain [7, 13, 20]. Although an initial report failed to find γ_6 expression in human cardiac tissue [7], microarray data (the Gene Expression Omnibus at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=geo) confirm the presence of γ_6 mRNA in human heart (Accession number: GDS651, GDS839) as well as human skeletal muscle (GDS264, GDS611). As was previously seen in rat [13], short isoforms of γ_6 lacking the second and third transmembrane domains are expressed in human tissue. The γ_6 subunit is the only member of the γ subunit family that is expressed as separate isoforms.

The four γ subunits identified as regulators of AMPA receptor function (γ_2, γ_3, γ_4, and γ_8; the TARPs) are widely expressed in the brain and share highly conserved sequences that are quite distinct from γ_1 and γ_6 [1, 4]. Notably, the cytoplasmic C-terminal regions of the TARPs contain a number of regulatory sites including a PDZ-binding motif. This PDZ-binding motif (TTPV) is critical for targeting AMPA receptors to the synapse.

The γ_5 and γ_7 subunits are also highly expressed in brain tissue but lack a C-terminal PDZ-binding motif [13, 37]. γ_5 and γ_7 are encoded by five exons in contrast to all other γ subunits, which are encoded by four exons [13, 37]. A protein originally identified as mouse γ_5 [30] is currently named transmembrane protein 37 (*Tmem37*, NCBI database). Although *Tmem37* is also a tetraspanin protein with the GLW motif characteristic of the pfam0082 protein family, its gene consists of only two exons, indicating a divergence from other γ subunit genes during evolution. Based on phylogenetic evidence, Chu et al. proposed that *Tmem37* should not be considered a member of the calcium channel γ family [13]. This conclusion is further supported by the observation that *Tmem37* does not share the chromosomal linkage to γ_4 and γ_1 in human [6], mouse and rat [13] that is seen for γ_5 (Table 2).

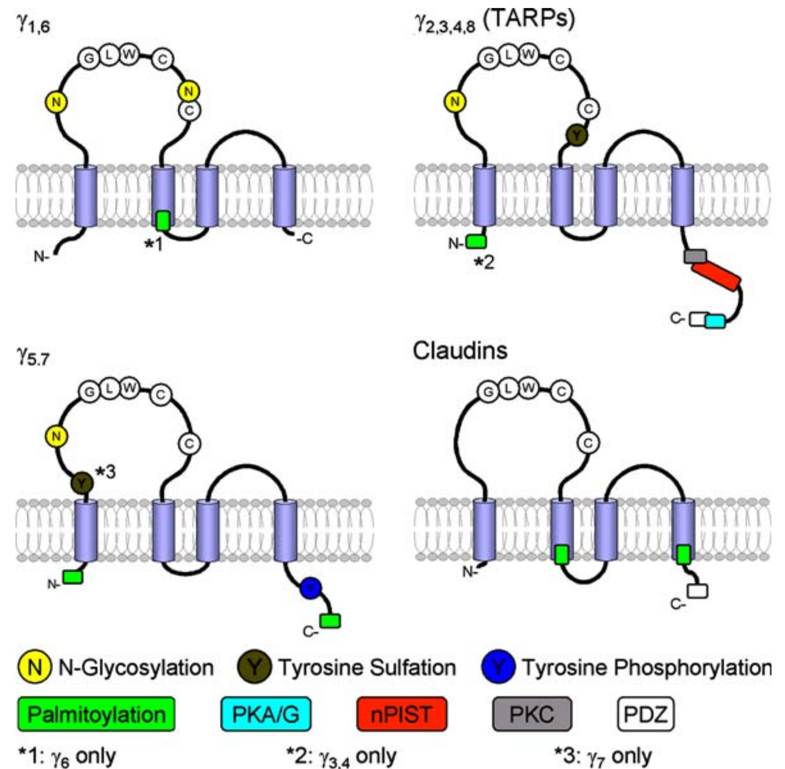

Fig. 1 Schematic diagrams showing predicted membrane topology and putative functional sites on γs and claudins. Only strongly conserved sites among rat, mouse and human are presented

Comparison of functional motifs within clusters of γ subunits

In addition to the critical PDZ-binding motif, the C-terminal regions of the four γ subunits known as the TARPs (γ_2, γ_3, γ_4, γ_8) also contain regulatory sites that control AMPA receptor targeting. For instance, the second threonine in the PDZ-binding motif (TTPV) of γ_2 is a consensus site for phosphorylation by PKA, PKC, PKG, and CaMKII suggesting that phosphorylation may regulate the interaction between γ_2 and the AMPA receptor. Indeed, two groups have independently found that phosphorylation by PKA interfered with the binding between γ_2 and PSD-95 [10, 12]. Consequently, reduced synaptic targeting of γ_2 prevents its interaction with the AMPA receptor, resulting in weakened synaptic efficiency [10]. Further elucidation of the regulation of γ subunits by intracellular phosphorylation or signaling may shed more light on the importance of γ subunits in receptor/channel trafficking within the cell.

Based on the observation that the TARPs contain conserved phosphorylation sites that modulate protein function, we wondered whether a comparison of functional sites between all of the γ subunits would provide an additional method of characterizing members of this protein family and might also reveal putative modulatory mechanisms that might be targets for future experimentation. We have extended the comparison of the distribution of regulatory and interaction sites within the γs by performing multiple sequence alignment and bioinformatic analysis on all the γ sequences from rat, mouse, and human[1]. Comparison of the distribution of putative modulatory sites within each γ subunit allowed us to group the γ subunits that contained identical sites at the same locations that were also conserved across species into the following clusters: (γ_1, γ_6), (γ_2, γ_3, γ_4, γ_8) and (γ_5, γ_7). Putative sites identified by this analysis that were found only in a single subunit are not included in this discussion. The clusters of putative functional sites identified by this analysis are identical to those derived previously from phylogenetic analysis and those derived from functional studies as discussed later. Thus, this analysis provides additional evidence supporting

[1] Published protein sequences of γ subunits and claudins were retrieved from NCBI protein database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein). The sequence accession numbers and chromosomal locations of γ genes used in this article are available in Tables 1 and 2, respectively. The hydropathy plot and transmembrane topology of γs were taken directly from Chu et al. [13]. The transmembrane topology of claudins was predicted by TMHMM program v2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Multiple sequence alignments were performed using ClustalW at default settings (http://www.ebi.ac.uk/clustalw/index.html). Putative functional sites in each sequence were identified in the online PROSITE database ([26], http://ca.expasy.org/prosite). Since no consensus motif of palmitoylation sites has been defined [48], in silico predictions based on experimental data in the literature were used to scan claudins and γs with the cut-off value of 2.6 [57].

Table 1 Accession numbers from NCBI protein database

Sequences	Human	Rat	Mouse
CACNG1	AAH69493	NM_019255	NP_031608
CACNG2	AAH69612	AF361339	NP_031609
CACNG3	AAH37899	AF361340	NP_062303
CACNG4	AAF14538	AF361341	NP_062304
CACNG5	AAL50046	AF361342	NP_542375
CACNG6	AAL50047	AF361343	NP_573446
CACNG7	AAL50048	AF361345	AAL50044
CACNG8	AAL50049	AF361346	NP_573453
TMEM37	NP_899063	NP_620795	NP_062305

Table 2 Chromosomal locations of calcium channel γ subunit genes and TMEM37

Sequences	Human	Rat	Mouse
CACNG2	22q13.1	7q33-q34	15E1
CACNG3	16p12-p13.1	1q36	7F2
CACNG1	17q24	10q32.1	11E1
CACNG4	17q24	10q32.1	11E1
CACNG5	17q24	10q32.1	11E1
CACNG6	19q13.4	1q12	7A1
CACNG7	19q13.4	1q12	7A1
CACNG8	19q13.4	1q12	7A1
TMEM37	2q14.2	13q11	1E2.3[a]

[a] Initially identified as CACNG5 by Klugbauer et al. [30]

significant diversity within the γ subunit family based on the existence of different functional motifs in the primary sequences (Fig. 1).

γ_2, γ_3, γ_4, γ_8: As mentioned previously γ_2, γ_3, γ_4, and γ_8 contain several common regulatory sites in both the extracellular and intracellular domains. The most distinct features of the TARPs are the terminal PDZ-binding motifs overlapped with PKA phosphorylation sites. The terminal TTPV motif is known to interact with PSD-95 in the post-synaptic density and the binding is regulated by the PKA motif immediately preceding the PDZ-binding motif [10, 12]. This combination determines the synaptic targeting of TARPs and AMPA receptors. In addition, the nPIST-binding motifs in the center portion of the C-terminal tail of TARPs regulates membrane-trafficking and synaptic targeting of the TARPs [14]. A series of nine serine residues in the N-terminal portion of nPIST-binding motif are regulated by kinases (CAMKII, PKC) and phosphatases (PP2B and PP1) [50]. A tyrosine sulfation site also exists in the first extracellular loop of the TARPs. Tyrosine sulfation occurs when proteins travel through the Golgi lumen. This modification strengthens protein–protein interactions and is usually observed in proteins involved in intercellular interactions and communication. Since the first extracellular loop of γ_2 has been implicated in the modulation of AMPA receptor gating, both the tyrosine sulfation and N-glycosylation sites would be interesting targets for mutation analysis.

γ_5, γ_7: γ_5, and γ_7 are palmitoylated at both the N- and C-termini. Additionally, their C-terminal tails contain tyrosine phosphorylation sites. A better understanding of the function of γ_5 and γ_7 is necessary before the significance of these sites can be determined.

γ_1, γ_6: All γs contain N-linked glycosylation sites in the first extracellular loop. However, only γ_1 and γ_6 have sites both before and after the signature GLW motif. Since the interaction between γ_1 and $Ca_v1.1$ is mapped to the first half of γ_1, the N43 and N80 residues of γ_1 might be good candidates for mutation analysis. Similarly these glycosylation sites may also be involved in the interaction between γ_6 and $Ca_v3.1$. γ_6 contains a unique palmitoylation site in the cytoplasmic end of the second transmembrane domain. Since palmitoylation increases membrane targeting of modified proteins [11, 54], γ_6 may have a different intracellular distribution than that of γ_1. Alternatively, dynamic palmitoylation of γ_6 may provide a functional switch for the modulation of VDCCs.

The distribution of putative phosphorylation sites within γ_1 and γ_6 is not highly conserved. The intracellular N-termini of γ_1 contains a putative PKC site and that of γ_6 contains a putative PKA site in mouse and rat but not in human. A casein kinase II site appears in the C-terminal tail of γ_1 in all three species but does not exist in γ_6. We have also analyzed the phosphorylation sites in γ_1 and γ_6 using a neural networked-based prediction [5] and reached a similar conclusion. In contrast to the numerous functional motifs in other γ subunits, the phosphorylation sites on γ_1 and γ_6 are scarce and mostly not conserved. It would appear that the physiological functions of γ_1 and γ_6 are not extensively regulated by protein phosphorylation. The short length (19–20 aa) of the C-terminal tails on γ_1 and γ_6 also preclude the existence of more than a few sites as targets of intracellular signaling. However, our analysis does not exclude the presence of novel functional motifs. Also, it is possible that γ_1 and γ_6 are indirectly regulated through interaction with other proteins that are targeted by cell signaling pathways.

γ Subunits perform multiple cellular functions

Three distinct cellular functions have been proposed for members of the γ subunit family including regulation of VDCC expression and function, regulation of AMPA receptor gating and trafficking and, most recently, regulation of cellular aggregation.

γ Subunit effects on VDCC electrophysiology

Much of our understanding of the effects of the γ subunits on calcium current come from studies in which channel subunits are over-expressed in heterologous cell lines or oocytes. Results from native cells are limited and sometimes contradictory to results obtained from heterologous systems.

γ_1, γ_6: The founding member of the γ subunit family, γ_1, was first isolated as a subunit of the high-voltage activated (HVA) calcium channel in skeletal muscle [27]. When co-expressed with the α_1 subunit $Ca_v1.2$ in heterologous systems, γ_1 alters HVA calcium current kinetics and the voltage-dependency of inactivation [16, 47], although a negative report demonstrating no effects of this subunit on calcium current has also been published [56]. The function of γ_1 has been studied in native skeletal muscle using the γ_1-null mouse [2, 19, 25]. Calcium current density in skeletal muscle from the knockout animal was significantly increased, confirming a physiological role for the γ_1 subunit as a negative regulator of calcium current in native cells. Detailed analysis concluded that the γ_1 subunit accelerates current inactivation [19], causes a hyperpolarizing shift in the voltage-dependence of inactivation and significantly reduces HVA calcium current density [2, 19, 25]. Interestingly, a change in calcium current density was observed only in juvenile knockout mice but not in adults, although the changes in voltage-dependence of inactivation were observed at both ages [25, 52]. This may indicate that the effects of γ_1 on voltage-dependence and current amplitude are independent of each other. Consistent with this idea, Held et al. [25] have shown that raising the intracellular cAMP level in wild-type myotubes mimics the effect of γ_1 deficiency on current amplitude in mutant myotubes, but not the effect on voltage-dependence of inactivation. Structurally, γ_6 is the closest homologue to γ_1. Since skeletal muscle also expresses $\gamma_{6,}$ and that γ_6 only affects calcium current density (see below), it is tempting to hypothesize that compensatory changes in expression of γ_6 may have partially mitigated the effects of the γ_1 knockout in the adult, although this hypothesis remains to be tested.

Co-expression of γ_6 with the α_1 subunit $Ca_v3.1$ in HEK293 cells significantly decreases the density of low-voltage activated (LVA) calcium current without significantly affecting current kinetics or voltage-dependency [22]. Both the γ_6 subunit and $Ca_v3.1$ are expressed in cardiac muscle at the same developmental stages [13, 33], therefore this pairing is potentially physiological. However, there have been no published studies describing the effects of γ_6 in native cells so the physiological significance of its reported effect on LVA current remains to be confirmed. Initial reports show that γ_6-null mice are viable without gross phenotypic alterations ([3], URL http://www.nih.gov/science/models/mouse/index.html). However electrophysiological analysis has not been reported using tissue from these animals.

Two studies have compared directly the effects of γ_1 and γ_6 on calcium current with those of TARPs in the same experimental systems. The results clearly demonstrate that the ability to decrease calcium current is a unique attribute of γ_1 and γ_6 that is not shared by members of the TARP cluster. Held and colleagues demonstrated that γ_2, a TARP, does not restore normal function in γ_1 knockout myocytes [25]. Likewise, γ_4, also a TARP, does not possess the inhibitory effect of γ_6 on LVA calcium current expressed in a cell line [22]. Based on these studies, chimeric γ_1 and γ_6 proteins, in which specific sequences are replaced by homologous sequences from TARP proteins, have been used to identify critical regions of γ_1 and γ_6 that are required for their ability to modulate calcium current. Studies with γ_1/γ_2 chimeras have identified the N-terminal half of the γ_1 subunit as necessary for its functional effects on and binding to HVA calcium channel [2]. Similar studies with γ_6/γ_4 chimeras confirm the importance of the N-terminal half of the protein and, in addition, have specifically identified the first transmembrane domain of γ_6 as containing a critical motif necessary for its ability to decrease LVA current density [23, 36].

γ_5, γ_7: There is little experimental evidence concerning the effect of γ_5 and γ_7 on calcium current. In one study, the γ_7 subunit dramatically reduced current density produced by the $Ca_v2.2$ subunit in Xenopus oocytes and COS-7 cells [37]. However, when expressed in sympathetic neurons γ_7 failed to affect pre-existing HVA Ca^{2+} channels. Therefore, confirmation of the physiological influence of γ_7 on calcium current remains elusive. As mentioned before, earlier studies on mouse γ_5 used a protein that is currently identified as *Tmem37* (Table 2). Interestingly, *Tmem37* did subtly accelerate calcium current activation and inactivation in heterologous expression system [30, 32].

γ_2, γ_3, γ_4, γ_8: A number of studies have investigated the electrophysiological effects on calcium current of the cluster of four γ subunits that are now known as TARPs. When over-expressed in heterologous systems γ_2, γ_3, and γ_4 are reported to hyperpolarize the voltage-dependence of inactivation of $Ca_v2.1$ (HVA) current by 3–7 mV [30, 34, 45]. However, the direction of this shift is apparently altered when a β subunit is co-expressed [45]. Two studies have reported small (3–5 mV) shifts in the voltage-dependence of activation by γ_2 but in different directions [30, 45]. The only reported effect of γ_2, γ_3, and γ_4 on $Ca_v3.3$ (LVA) current is that γ_2 slows the deactivation rate of $Ca_v3.3$ channel [21]. Unlike the results with γ_1 and $\gamma_{6,}$ significant decreases in current amplitude were not routinely found with any of the TARPs, although modulation

of Ca_v2 current amplitude has been reported [28]. Currently, there is no evidence supporting an effect of these subunits on calcium currents in native cells. Specifically, there are no observable changes in calcium currents recorded from cerebellar granule neurons in the stargazer mouse in which the γ_2 gene is mutated [9].

Of the eight γ subunits, γ_1 and γ_6 have the most significant effects on current density when co-expressed with calcium channel α_1 subunit. The idea that γ_1 and γ_6 act as specific regulators of calcium current is supported by studies that directly compared the effects of γ_1 and γ_6 on calcium current with those of representative members (γ_2 and γ_4, respectively) of the TARP subfamily of γ subunits [2, 22, 36]. When direct comparisons of this type were made it was found that while γ_1 and γ_6 were potent modulators of calcium current density, the TARP proteins had no effect when co-expressed under identical experimental conditions. These results support the idea that the historical role proposed for γ_1, and later for γ_6, as a calcium channel subunit that modifies current is correct, but this is a function not shared by all members of the γ subunit family, specifically the TARPs.

Effects on AMPA receptor trafficking and gating

The second member of the calcium channel γ subunit family, γ_2, was identified from genetic analysis of the stargazer mouse, an animal that displays a characteristic phenotype that includes head tossing and epileptic episodes [39]. Sequence analysis of the mutated gene, stargazin, revealed a homology to γ_1, and stargazin (γ_2) was thus initially identified as a VDCC auxiliary subunit. However, analysis of the stargazer mouse showed that cerebellar mossy fiber synaptic transmission was disrupted in these animals due to reduced synaptic AMPA receptors in the granule cells [8, 24]. Transfecting γ_2 into γ_2-null granule cells rescues the AMPA response in theses neurons [9] and it was subsequently found that γ_2 mediated membrane trafficking and synaptic targeting of the GluR1, GluR2, and GluR4 subunits of the AMPA receptor. As additional homologues to γ_1 were discovered, their ability to alter calcium channel and AMPA receptor function were characterized. Using AMPA receptor clustering as an assay, Tomita et al. [49] demonstrated that the γ_2, γ_3, γ_4, and γ_8 subunits share a common function as transmembrane AMPA receptor regulatory proteins. Vandenberghe et al. [55] have demonstrated that γ_2 is tightly associated with AMPA receptors in cerebellar extract. Under basal condition, γ_2 was never found without bound AMPA receptor suggesting that γ_2 is an auxiliary subunit of AMPA receptors.

It appears that TARPs can modulate the biophysical properties of AMPA receptor currents, as well as regulate the trafficking of AMPA receptors in neurons. Specifically, the γ_2 subunit has been shown to enhance AMPA receptor current by slowing its desensitization and deactivation, and probably also increasing the rate of channel opening [42, 50]. The first extracellular loop and second transmembrane domain of γ_2 are required for this biophysical modulation of AMPA current [50]. As for AMPA receptor trafficking, it appears that TARPs control AMPA receptor membrane-trafficking and synaptic targeting through several distinct steps. It has been suggested that γ_2 associates with GluR subunits early on during receptor biogenesis at the level of ER/Golgi [49]. Subsequently, nPIST, a Golgi-resident PDZ protein, binds residues 243–283 in the C-terminal tail of γ_2 [14]. Binding of nPIST to γ_2/AMPAR complex allows nPIST to act as a chaperone during the membrane-trafficking process. Once in the plasma membrane, it is proposed that an interaction between nPIST and PSD-95 mediates the translocation of γ_2/AMPAR complex from an extrasynaptic locus to underneath the synapse [14]. Finally, interaction of PSD-95 and related proteins with the PDZ-binding motif (TTPV) of γ_2 and GluR subunits anchors the γ_2/AMPAR complex in the post-synaptic density, completing the synaptic targeting process [46]. Phosphorylation of the second threonine of the PDZ-binding motif disrupts γ_2 interaction with PSD-95 and downregulates synaptic AMPA current [10, 12]. It also should be noted that the nPIST-binding motif is present in all of the TARPs [14], with nine conserved serine residues around the N-terminal region of this sequence. Phosphorylation and dephosphorylation of these serines dynamically regulate synaptic AMPA receptor current, suggesting an important role of TARPs in synaptic plasticity [51].

There is evidence that members of the TARPs are functionally interchangeable. Transgenic mice lacking γ_4 have normal phenotypes [3]. However, breeding the γ_4 mutant mice onto a γ_2 mutant background makes the double mutants more seizure prone than γ_2 single mutants [35]. This result supports the idea that the TARPs have overlapping functions and may be able to functionally compensate for each other. Conversely, in cells where one TARP isoform predominates, the compensation may not be complete. Hippocampal CA1 cells normally show robust expression of the γ_8 subunit although mRNAs encoding other γ subunits are also expressed [20]. Using γ_8-null mice, Rouach et al. [44] demonstrated that GluR2/3 expression is reduced and long-term potentiation impaired in hippocampal CA1 cells although neither process is totally absent. In fact, all four TARPs have been proposed to play important roles in synaptic plasticity based on their ability to regulate AMPA receptors [51].

It should be emphasized that neither γ_1 nor γ_5 alters AMPA receptor trafficking in cerebellar granule cells from γ_2 null mouse [49], which is consistent with the idea that γ_1

and γ_5 cannot function as regulators of AMPA receptor targeting. This result is not unexpected since the C-terminal regions of γ_1 and γ_5 (as well as those of γ_6 and γ_7) do not contain PDZ-binding motifs and therefore lack the ability to interact with anchoring proteins.

Do γ subunits act as adhesion molecules?

Recently, it was suggested that at least some γ subunits may be involved in cell–cell interactions. When γ_2 is transfected into mouse L-fibroblasts, which normally do not form cell–cell aggregates, the cells start to exhibit cell–cell adhesion [41]. An N-glycosylation site (N48) on the first extracellular loop of γ_2 is critical for this junctional interaction. The intercellular aggregation is indistinguishable from that seen when L-cells are transfected with an orthodox junctional protein, claudin 1. If confirmed, this very interesting and novel observation suggests yet another role for members of the γ subunit family.

Conclusion

Contrary to what their name implies, the eight members of the calcium channel γ subunit family represent a functionally diverse group of proteins. Experimental, phylogenetic, and bioinformatic analyses support the existence of three distinct clusters within the family: (γ_1, γ_6), (γ_5, γ_7) and (γ_2, γ_3, γ_4, γ_8; TARPs). Of these the TARPs have received the most experimental attention given their recently described ability to mediate AMPA receptor trafficking and to modulate glutamate current kinetics as first revealed by the stargazer mouse. However, the evidence that γ_1 and γ_6 do in fact regulate VDCC current (and, importantly, do not regulate AMPA receptors) is compelling. The physiological function of γ_5 and γ_7 remains largely unexplored. The members of each cluster contain conserved regulatory motifs that help differentiate the groups. The presence or absence of conserved PDZ-binding motifs, tyrosine sulfation sites, PKA sites, N-glycosylation, and palmitoylation sites suggest divergent intracellular regulation and function. The novel demonstration that γ_2 affects cell aggregation suggests that we may not have uncovered all of the cellular functions of these proteins.

Many important questions remain. In some cases direct demonstration of effects of specific γ subunits in native cells is not available limiting our understanding of the impact of these proteins on cell function. This is especially true for the cluster including γ_5 and γ_7. In addition, much remains to be understood concerning the details of the molecular interactions of the γ subunits with their binding partners. Since many cells express multiple γ subunit isoforms, delineating the precise roles played by each will be challenging. Given the number and diversity of regulatory motifs identified in the proteins, the full extent of intracellular signaling pathways as modifiers of γ subunit function is yet to be determined. Finally, it will be interesting to see what additional roles, like regulating cell aggregation, these multi-talented proteins may play.

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