Localization and Enhanced Current Density of the Kv4.2 Potassium Channel by Interaction with the Actin-Binding Protein Filamin

Kevin Petrecca, David M. Miller, and Alvin Shrier

Department of Physiology, McGill University, Montréal, Québec, Canada H3G 1Y6

Kv4.2 potassium channels play a critical role in postsynaptic excitability. Immunocytochemical studies reveal a somatodendritic Kv4.2 expression pattern, with the channels concentrated mainly at dendritic spines. The molecular mechanism that underlies the localization of Kv4.2 to this subcellular region is unknown. We used the yeast two-hybrid system to identify the Kv4.2-associated proteins that are involved in channel localization. Here we demonstrate a direct interaction between Kv4.2 and the actin-binding protein, filamin. We show that Kv4.2 and filamin can be coimmunoprecipitated both in vitro and in brain and that Kv4.2 and filamin share an overlapping expression pattern in the cerebellum and cultured hippocampal neurons. To examine the functional consequences of this interaction, we expressed Kv4.2

in filamin+ and filamin− cells and performed immunocytochemical and electrophysiological analyses. Our results indicate that Kv4.2 colocalizes with filamin at filopodial roots in filamin+ cells but shows a nonspecific expression pattern in filamin− cells, with no localization to filopodial roots. Furthermore, the magnitude of whole-cell Kv4.2 current density is ~2.7-fold larger in filamin+ cells as compared with these currents in filamin− cells. We propose that filamin may function as a scaffold protein in the postsynaptic density, mediating a direct link between Kv4.2 and the actin cytoskeleton, and that this interaction is essential for the generation of appropriate Kv4.2 current densities. 

Key words: Kv4.2; potassium channels; filamin; postsynaptic density; subcellular localization; actin-binding protein

Appropriate synaptic transmission is dependent on the precise localization of ion channels and neurotransmitter receptors at specific subcellular sites (Sheng, 1996; Ziff, 1997; Collodge and Froehner, 1998; Craven and Bretd, 1998). Voltage-gated K+ channels are expressed within neurons in a variety of spatial distributions (Sheng et al., 1992; Wang et al., 1994; Veh et al., 1995) where they function as regulators of membrane excitability and synaptic transmission (Hille, 1991; Magee et al., 1998). Fast transient (A-type) K+ channels, members of the voltage-gated K+ channel family that are found in a wide variety of excitable cells, have been implicated in the control of action potential frequency and threshold, action potential configuration, neurotransmitter release, and postsynaptic excitability (Jan and Jan, 1997; Magee et al., 1998). Kv1.4 and Kv4.2, fast transient K+ channel family members, are segregated differentially in neurons. Kv1.4 is localized to axons exhibiting a concentration at the presynaptic terminal (Sheng et al., 1992; Zito et al., 1997; Arnold and Clapham, 1999), whereas Kv4.2 is localized to the somatodendritic compartment exhibiting a concentration at the postsynaptic terminal (Sheng et al., 1992; Maletic-Savatic et al., 1995; Alonso and Widmer, 1997).

In this study we set out to identify Kv4.2-associated proteins that are involved in Kv4.2 localization. Here we report the identification and characterization of a novel interaction between Kv4.2 and filamin, a member of the α-actinin/spectrin/dystrophin family of actin-binding proteins. Filamin originally was identified as a protein isolated from motile alveolar macrophage that caused purified actin-binding proteins. Filamin originally was identified as a protein isolated from motile alveolar macrophage that caused purified actin-binding proteins. Filamin was later shown to be a specific subcellular site (Sheng, 1996; Ziff, 1997; Colledge and Froehner, 1998).

Here we demonstrate a direct interaction between Kv4.2 and the actin-binding protein, filamin. We show that Kv4.2 and filamin can be coimmunoprecipitated both in vitro and in brain and that Kv4.2 and filamin share an overlapping expression pattern in the cerebellum and cultured hippocampal neurons. To examine the functional consequences of this interaction, we expressed Kv4.2

in filamin+ and filamin− cells and performed immunocytochemical and electrophysiological analyses. Our results indicate that Kv4.2 colocalizes with filamin at filopodial roots in filamin+ cells but shows a nonspecific expression pattern in filamin− cells, with no localization to filopodial roots. Furthermore, the magnitude of whole-cell Kv4.2 current density is ~2.7-fold larger in filamin+ cells as compared with these currents in filamin− cells. We propose that filamin may function as a scaffold protein in the postsynaptic density, mediating a direct link between Kv4.2 and the actin cytoskeleton, and that this interaction is essential for the generation of appropriate Kv4.2 current densities.

Materials and Methods

Yeast two-hybrid screen and analysis of Kv4.2–filamin interaction. Yeast two-hybrid screens were performed with the Y190 yeast strain harboring the reporter genes HIS3 and β-galactosidase (β-gal) under the control of upstream gal4-binding sites (Clontech Laboratories, Palo Alto, CA). The Kv4.2C1 bait was generated by incorporating unique Ncol and Xbal restriction sites 5′ and 3′, respectively, via PCR and was fused to the gal4 DNA-binding domain in vector pAS2-1. This bait, pAS2-1/Kv4.2C1, was used to screen ~3×106 clones from a human heart cDNA library constructed in the GAL4-activation domain vector pACT-2 (Clontech). Deletion variants of pAS2-1/Kv4.2C1 were constructed by PCR with the use of specific primers and were subcloned into pAS2-1 for yeast two-hybrid interactions. Mutations of pAS2-1/Kv4.2C1 were generated by using QuikChange (Stratagene, La Jolla, CA). The Kv4.3C bait was generated in a manner similar to Kv4.2C1. The HRCG bait was generated by incorporating unique Ncol and BamHI restriction sites 5′ and 3′, respectively, via PCR and was fused to the gal4 DNA-binding domain in vector pAS2-1. Expression constructs. The GST–Kv4.2(aa 471–630) fusion construct was generated by digesting pAS2-1/Kv4.2C1 with BamHI and SmaI (aa 471–630) and subcloning it into pGEX-2T (Pharmacia, Piscataway, NJ). pCMV/myc–Kv4.2 was generated by subcloning full-length Kv4.2 into pCMV-myc (Stratagene). pSG-5/HA–filamin (filaminC, aa 2172–2705) was generated by subcloning the original pACT-2 library clone, containing
Neuron culture and immunocytochemistry. Low-density hippocampal neuronal cultures were prepared from hippocampi dissected from 3-d-old Sprague Dawley rats and stored in an oxygenated solution. Then the hippocampi were enzymed with the absence of [35S] and used for the binding assay. The membrane was incubated with 10 μCi [35S]filamin overnight at 4°C. The next day the membrane was brought to room temperature, washed, immunoblotted with anti-HA; enhanced chemiluminescence was performed with the ECL detector kit (Amersham). Then the membrane was dried and submitted for autoradiography.

RESULTS

Interaction of Kv4.2 with filamin

In an effort to search for molecules that may be involved in the localization of Kv4.2, we used the entire C terminus of Kv4.2 as bait to screen a human heart cDNA library, using the yeast two-hybrid system. The screen yielded multiple copies of four distinct cDNAs encoding polypeptides that interacted specifically with the C terminus of Kv4.2. No other clones were isolated in this screen. Furthermore, an unrelated bait encoding the C terminus of HERG did not interact with any of the isolated clones. Sequence analysis revealed that one of the cDNAs was derived from filamin A, whereas the other three cDNAs were derived from distinct but overlapping sequences of a highly homologous polypeptide, filamin C (Fig. 1A). Three filamin isoforms (A, B, and C) have been identified, exhibiting an overall amino acid homology of 70–72%. Each isoform shares three common functional domains: a N-terminal actin-binding domain that is structurally similar to that of the a-actinin/spectrin/dystrophin family of cytoskeletal proteins, a semiflexible rod domain composed of 24 repeats interrupted by two short sequence inserts of 20–40 aa between repeats 15–16 and 23–24, and a C-terminal self-association domain (Xie et al., 1998). All partial filamin cDNA fragments that were isolated in this screen began at variable starting points within repeat 20 and were complete to the C terminus (Fig. 1A).

To define the site of interaction between Kv4.2 and filamin, we

McKv4.2, myc-Kv4.2/600, and myc-Kv4.2/ATAA were in vitro-translated in the presence of [35S] methionine, electrophoresed, transferred, and renatured as above. P5G/5-HA–filamin C (aa 2172–2705) was in vitro-translated in the absence of [35S] and used for the binding assay as above. The next day the membrane was brought to room temperature, washed, immunoblotted with anti-HA; enhanced chemiluminescence was performed with the ECL detector kit (Amersham). Then the membrane was dried and submitted for autoradiography.
Figure 1. The domain structure of filamin and interaction with Kv4.2. A. Human cDNA clones isolated with a yeast two-hybrid screen, using the Kv4.2 C-terminal region (aa 395–630) as bait, are shown aligned below a schematic representation of the filamin domain structure. **ABD**, Actin binding domain; **1–15, 16–23, and 24** represent 96 aa repeats, each separated by hinge regions. Partial cDNAs from filaminA and filaminC genes were isolated. The numbers in parentheses indicate the number of times each clone was isolated with the yeast two-hybrid screen. B, Sequence requirements in the Kv4.2 C-terminal region for interaction with filamin. FilaminC (aa 2172–2705), binding to Kv4.2C1 (aa 395–630), and deletion derivatives were assayed by HIS3/β-gal induction in the yeast two-hybrid system. Residues 601–604 are required for interaction with filamin; deletion and/or mutation of this region abolishes the interaction. Kv4.3, which contains the identical binding region, also interacts with filamin. The HERG C-terminal region (aa 864–1165) does bind filamin. The various bait fragments were tested for filamin binding by semiquantitative yeast two-hybrid interaction assays that were based on the degree of induction by the reporter genes HIS3 and β-gal. HIS3 activity was measured by the percentage of colonies growing on histidine-lacking medium as compared with the full-length Kv4.2 bait (Kv4.2C1): ++++, >75%; ++, >50%; +, >25%. β-Gal activity was determined from the time that was taken for the colonies to turn blue in X-gal filter lift assays performed at room temperature: ++++, <2 hr; ++, <3 hr; +, <4 hr; −, no significant activity. **H6**, Sixth transmembrane domain.
began by examining successively larger C-terminal deletions of Kv4.2 that bind filamin, using yeast two-hybrid analysis. Deletion of the C-terminal 25 aa did not affect binding (Fig. 1B). However, deletion of the next four amino acids (601–604) completely abolished the interaction, suggesting that these amino acids (PTPP) are necessary for Kv4.2 interaction with filamin. We next generated point mutations within this region, using the entire C-terminal Kv4.2 bait fragment (Kv4.2C1). Substitution of the prolines in the 601–604 aa region to alamines (PTPP→ATAA) completely abolished the interaction (Fig. 1B). These observations indicate that this proline-rich region is a domain that is necessary for Kv4.2 interaction with filamin. This sequence was noted to be identical in Kv4.3, consistent with its representing a site of interaction with both members of the Kv4 family. The subsequent use of yeast two-hybrid analysis confirmed that filamin also interacts with Kv4.3 (Fig. 1B).

Association of Kv4.2 and filamin in situ and in vitro

To investigate the interaction of Kv4.2 and filamin further, we tested whether these proteins form a complex in transfected heterologous cells. COS7 cells, transfected either singly or doubly with HA epitope-tagged filamin (HA–filamin) and myc-tagged Kv4.2 (myc-Kv4.2), myc-Kv4.2/600, or myc-Kv4.2/ATAA, were solubilized and immunoprecipitated with an anti-HA antibody. The immunoprecipitates were resolved by SDS-PAGE and transferred to a PVDF membrane. The proteins were immunoblotted with anti-filamin (Fig. 2A) and anti-Kv4.2 antibodies. The identical blot was stripped and reprobed with control IgG (Fig. 2A), Sigma; lane 5, Serotec) specifically precipitate Kv4.2, as visualized by blotting with an anti-Kv4.2 antibody. Immunoprecipitation with control IgG (lane 5) does not pull down Kv4.2, demonstrating the specificity of the immunoprecipitation and competition of the anti-Kv4.2 antibody; the immunogenic peptide completely blocked the labeling of Kv4.2 (lane 4). The Input lane was loaded with 5% of the extract used for immunoprecipitation (lane 3). B, The immunoblot shows that two different anti-filamin antibodies (lane 2, Sigma; lane 3, Serotec) do not pull down HERG, as visualized by blotting with an anti-HERG antibody. The Input lane was loaded with 5% of the extract that was used for immunoprecipitation (lane 1).

Figure 2. Coimmunoprecipitation in heterologous cells and direct binding of Kv4.2 and filamin. A, Extracts from COS7 cells singly or doubly transfected with HA–filamin and myc-Kv4.2, myc-Kv4.2/600, myc-Kv4.2/ATAA, or myc-HERG were immunoprecipitated with anti-HA antibodies. The immunoprecipitates were immunoblotted with anti-myc (top panel) and anti-HA antibodies (bottom panel). B, Extracts from Kv4.2-transfected filamin and filamin cells were immunoprecipitated with anti-filamin antibodies and immunoblotted with anti-filamin (top panel) and anti-Kv4.2 (bottom panel) antibodies. C, Filter overlay assay showing direct in vitro binding of [35S]filamin to Kv4.2. Glutathione S-transferase (GST) and GST-Kv4.2 (aa 417–630) fusion proteins were prepared as crude bacterial lysates and were purified with glutathione-Sepharose beads. Protein (5 μg) was resolved by SDS-PAGE and transferred to a PVDF membrane. Top panel, Renatured membrane overlaid with [35S]filamin showing specific binding to GST-Kv4.2. Bottom panel, Ponceau S-stained membrane showing the position and similar abundance of proteins in each lane. D, Filter overlay assay showing direct in vitro binding of HA-filamin to in vitro-translated Kv4.2, but not to Kv4.2/600 nor Kv4.2/ATAA. In all, 15 μl of in vitro-translated [35S]Kv4.2, [35S]Kv4.2/600, and [35S]Kv4.2/ATAA was resolved by SDS-PAGE and transferred to a PVDF membrane. Top panel, Renatured membrane overlaid with in vitro-translated HA-filamin and immunoblotted with anti-HA showing specific binding to Kv4.2, but not to Kv4.2/600 nor Kv4.2/ATAA. Bottom panel, The identical blot was stripped and exposed to autoradiography, showing the position and similar abundance of the in vitro-translated protein products in each lane.

The in vitro overlay assay that used a GST–Kv4.2 bacterial fusion protein and in vitro-translated [35S]filamin (Fig. 2C). A purified GST–Kv4.2 (aa 471–630) fusion protein and GST alone were separated by SDS-PAGE and transferred to a PVDF membrane. The proteins were renatured on the membrane and probed with in vitro-translated [35S]filamin. Filamin bound to the GST–Kv4.2 fusion protein but did not bind GST alone (Fig. 2C, top panel). Moreover, when the overlay assay was performed on non-renatured membranes, the interaction was abolished, indicating that Kv4.2 must be in a native state to interact with filamin.
conformation for association with filamin. To demonstrate the specificity of this interaction further, we separated in vitro-translated [35S]Kv4.2, [35S]Kv4.2/600, and [35S]Kv4.2/ATAA by SDS-PAGE and transferred them to a PVDF membrane. The proteins were renatured on the membrane and probed with in vitro-translated HA–filamin. Figure 2D (top panel) shows that, on immunoblotting with an anti-HA antibody, filamin specifically interacts with Kv4.2 (lane 1, top panel), but not Kv4.2/600 (lane 2, top panel) nor Kv4.2/ATAA (lane 3, top panel). Taken together, these data show that Kv4.2 and filamin form a complex in heterologous cells and that they interact directly in an in vitro assay.

**Association of Kv4.2 and filamin in vivo**

To determine whether Kv4.2 and filamin interact in vivo, we performed coimmunoprecipitation experiments from rat cerebellum. Membrane fractions from rat cerebellar homogenates were solubilized, and the supernatant was immunoprecipitated with two distinct anti-filamin antibodies. The immunoprecipitates were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with an anti-Kv4.2 antibody (Fig. 3A). A band at ∼74 kDa is seen with anti-Kv4.2 immunoblotting, indicating that filamin is able to coimmunoprecipitate Kv4.2 (Fig. 3A, lanes 1, 2). Kv4.2 was not precipitated when control IgG was used as the precipitating antibody (lane 5), indicating the specificity of the coimmunoprecipitation. Moreover, competition of the anti-Kv4.2 antibody with the immunogen peptide completely blocked the labeling of Kv4.2 band (lane 4). As a further control demonstrating the specificity of this interaction, we determined whether filamin was also capable of immunoprecipitating HERG. Figure 3B shows that, although HERG was readily detectable in brain lysates (Fig. 3B, lane 1), no HERG could be immunoprecipitated with either anti-filamin antibody (Fig. 3B, lanes 2, 3). These data demonstrate that Kv4.2 and filamin form a complex in brain.

**Kv4.2 colocalizes with filamin in cultured hippocampal neurons**

The results described above show that Kv4.2 and filamin interact both in vitro and in brain. Do filamin and Kv4.2 colocalize in neurons? This question was addressed by determining the localization of endogenous Kv4.2 and filamin in cultured hippocampal neurons. As shown in Figure 4, A–D, Kv4.2 exhibits a punctate staining pattern along dendrites, matching closely that of the presynaptic marker synaptophysin. Colabeling of endogenous Kv4.2 and endogenous filamin shows that filamin colocalizes with Kv4.2 in dendrites with an enrichment at synapses (Fig. 4E,F). Thus, at the light microscope level, filamin colocalizes with Kv4.2 in dendrites.

**Kv4.2 and filamin colocalize in cerebellum**

Kv4.2 immunoreactivity is present at high levels in the granule cell layer of the cerebellar cortex, exhibiting a somatodendritic localization (Sheng et al., 1992). As such, we used high-resolution confocal imaging to determine whether Kv4.2 and filamin colocalize in cerebellar sections, using a previously characterized anti-Kv4.2 antibody (Barry et al., 1995). Figure 5A shows that Kv4.2 is expressed abundantly in the cerebellar granule cell layer, consistent with Sheng et al. (1992), and that there is an overlap between Kv4.2 and filamin immunoreactivity in this cell layer. To determine whether this colocalization represents a synaptic localization, we determined whether Kv4.2 immunoreactivity correlated with that of the synaptic marker synaptophysin. Figure 5B shows that the Kv4.2 expression pattern highly correlates with that of synaptophysin. Thus, at the light microscope level, these data show that Kv4.2 and filamin share an overlapping expression pattern in the granule cell layer of the cerebellum, consistent with the direct association of these proteins in vivo.
Filamin localizes Kv4.2 to filopodial roots in heterologous cells

To examine whether filamin colocalizes with Kv4.2 in heterologous cells and to determine the significance of this interaction on Kv4.2 localization, we transfected myc-Kv4.2 into a filamin-deficient human malignant melanoma cell line (M2) and a M2 cell line stably expressing filamin (Cunningham et al., 1992) and analyzed their localization immunocytochemically. Figure 6, A and B, shows that myc-Kv4.2 accumulates and colocalizes with filamin in filamin + M2 cells at the roots of filopods. Filamin localization at the roots of filopods is consistent with a previous report demonstrating the role of filamin in the induction of filopodia (Ohta et al., 1999). In contrast, Kv4.2 expression in filamin − M2 cells shows a more uniform expression pattern, with no localization at filopodial roots (Fig. 6G,H). To determine whether the loss of Kv4.2 localization at filopodial roots is directly attributable to the absence of the filamin interaction or to a general loss of filamin expression, we transfected nonfilamin-interacting myc-Kv4.2/600 and myc-Kv4.2/ATAA mutant channels into filamin + M2 cells. Similar to wild-type Kv4.2 distribution in filamin + M2 cells, neither myc-Kv4.2/600 nor myc-Kv4.2/ATAA colocalizes with filamin in filamin + M2 cells, but each shows a more uniform expression pattern with no specific localization (Fig. 6C–F). These data demonstrate that Kv4.2 colocalizes with filamin in heterologous cells and that the accumulation of Kv4.2 at filamin-rich filopodial roots is dependent on its interaction with filamin.

Effect of Kv4.2–filamin association on whole-cell Kv4.2 current density

To determine the significance of the Kv4.2–filamin association on whole-cell Kv4.2 current, we recorded, using the patch-clamp technique, the current generated from Kv4.2-transfected filamin + and filamin − cells in whole-cell clamp mode. As is evident from the recordings, there is a prominent transient outward current present in Kv4.2-transfected cells in the presence or absence of filamin.

However, the current density, measured from the initial transient outward peak to the current level at the end of the 500 msec step, was 2.6-fold greater in the filamin + than in the filamin − cells (Fig. 7A,B). This also is reflected in the bar graph (Fig. 7F). These results demonstrate that Kv4.2 channels are expressed functionally and suggest that their interaction with filamin enhances the current density. To determine whether this difference in whole-cell current density was directly attributable to the Kv4.2–filamin interaction or to a nonspecific lack of filamin in filamin − cells, we expressed the Kv4.2/ATAA nonfilamin-interacting mutant channel in filamin + cells. As shown in Figure 7C, the magnitude of the whole-cell Kv4.2/ATAA current density was 2.8-fold less than that of wild-type Kv4.2 expressed in filamin + cells and similar to that of wild-type Kv4.2 expressed in filamin − cells. This is reflected in the bar graph (Fig. 7F). It is noteworthy that the Kv4.2/ATAA currents had markedly slower inactivation kinetics as compared with wild-type Kv4.2, most likely resulting from a nonfilamin-related effect on C-type channel inactivation. Experiments conducted with untransfected or mock-transfected cells never expressed a transient outward current. Instead, a small, relatively rapidly activating endogenous delayed rectifier current generally was observed that did not inactivate during the 500 msec step (see Fig. 6D,E). The same endogenous current also could be revealed in cells expressing Kv4.2 current when the Kv4.2 channels were inactivated by changing the surface membrane or to a change in the single-channel conductance, we assessed the single-channel conductance of Kv4.2 expressed in filamin + cells and similar to that of.

To determine whether the ~2.7-fold increase in whole-cell Kv4.2 current density is attributable to a higher density of channels in the surface membrane or to a change in the single-channel conductance, we assessed the single-channel conductance of Kv4.2 expressed in each of these cell lines. In agreement with previous studies (Cooper and Shrier, 1985; Cooper and Shrier, 1989), we found single-channel conductances with high K+ concentration in the pipette medium to be 46.0 ± 1.79 pS (n = 5) and 43.6 ± 1.33
pS (n = 5) in filamin+ and filamin− cells, respectively (data not shown). This difference was determined not to be statistically significant. As a further control we transfected the HERG K+ channel into filamin+ (n = 4) and filamin− (n = 4) cells and recorded the magnitude of the whole-cell current. Our results revealed no difference in current density between these cell lines (data not shown). Moreover, total cellular Kv4.2 protein expression is equivalent in filamin+ and filamin− cells (see Fig. 2B, bottom panel). Taken together, the electrophysiological data reveal that Kv4.2 is expressed in the surface membrane in the presence or absence of the filamin interaction; however, the presence of the interaction results in an increase in the density of Kv4.2 channels that are expressed in the surface membrane.

**DISCUSSION**

Here we report a novel protein–protein interaction between Kv4.2 and filamin, a member of the α-actinin/spectrin/dystrophin family of actin-binding proteins. We have defined a PTPP motif in the C terminus of Kv4.2 (aa 601–604) that is required for filamin interaction. Deletion or mutation of this motif abolishes the interaction, as determined by the yeast two-hybrid assay. We also show that Kv4.3, which contains the identical C-terminal tail PTPP motif, also interacts with filamin in the yeast two-hybrid assay. In addition, we demonstrate that Kv4.2 and filamin directly interact in in vitro assays and can be coimmunoprecipitated from heterologous cells and rat brain extracts. Furthermore, immunolabeling experiments reveal that Kv4.2 and filamin share an overlapping expression pattern in the cerebellum and cultured hippocampal neurons.

Immunocytochemical analyses show that Kv4.2 is enriched and colocalizes with filamin at cellular specializations: the roots of filopods in filamin+ heterologous cells. Deletion or mutation of the Kv4.2-binding motif abolishes this enrichment and colocalization, indicating that filamin plays a role in Kv4.2 localization. Expression of Kv4.2 in filamin+ cells also results in a loss of Kv4.2 localization at filopodial roots. Moreover, the magnitude of whole-cell Kv4.2 current density is ~2.7-fold larger in filamin+ cells than in filamin− cells as compared with these currents in filamin− cells. The combined immunocytochemical and electrophysiological data indicate that the observed difference in the magnitude of whole-cell Kv4.2 current density most likely is attributable to an increase in the number of functional channels in the surface membrane as opposed to a change in the single-channel conductance of Kv4.2. Taken together, these findings indicate that filamin is a Kv4.2-interacting cytoskeletal protein that colocalizes with Kv4.2 in neurons and plays an important role in the localization and functional surface membrane of Kv4.2 in heterologous cells.

**What determines filamin localization?**

The data presented in this study and others indicate that filamin exhibits a restricted distribution within the cell, localized primarily at cellular specializations, e.g., focal adhesion sites (Burridge and Chrzanowska-Wodnicka, 1996; Schwarzman et al., 1999) and filopodial roots (Ohta et al., 1999). This restricted localization may...
be attributable to its interaction with the adhesion molecule integrin, a constituent protein of cellular specializations (Burridge and Chrzanowska-Wodnicka, 1996). Interestingly, both integrin and filamin have been implicated as molecular components of the neuromuscular junction (NMJ), and β1-integrin has been shown to play a physiological role in the agrin-mediated signaling cascade that leads to AChR clustering (Meier and Wallace, 1998). Similarly, filamin has been implicated in stabilizing AChR clustering at the NMJ (Shadiack and Nitkin, 1991) and recently has been shown to interact directly with sarcoglycan (Thompson et al., 2000).

**Role of filamin at the synapse?**

This study demonstrates that filamin colocalizes and interacts with Kv4.2 in neurons. Using the yeast two-hybrid assay, we mapped the filamin interaction site on Kv4.2 to a proline-rich region (PTPP) at aa 601–604. This PTPP motif constitutes a consensus SH3-binding module (Pawson and Scott, 1997); however, no SH3 domains were found within filamin. Alternatively, the dependence of binding on prolines suggests a role for these amino acids in establishing the appropriate secondary structure that is required for Kv4.2–filamin interaction. A similar proposal has been made for the interaction between group 1 metabotropic glutamate receptors and Homer proteins (Tu et al., 1998).

The postsynaptic localization of Kv4.2 is consistent with the involvement of this fast-transient K⁺ channel in regulating the excitability of the postsynaptic membrane and thus the reception and integration of synaptic signals (Sheng et al., 1992; Alonso and Widmer, 1997). The combined immunocytochemical and electrophysiological findings presented here support two overlapping roles for filamin with respect to Kv4.2 binding: surface membrane expression and subcellular localization of Kv4.2.

In heterologous cells, filamin is necessary for the induction of filopodia (Ohda et al., 1999). A model has been put forth in which dendritic spine formation results from the induction of filopod-like dendritic spine precursors under synaptic boutons on axons (Matus, 1999). Filamin also has been demonstrated to exist in two intracellular pools in a phosphorylation-dependent manner: one associated with the plasma membrane and the other within the actin cytoskeletal network (Sharma et al., 1995; Meyer et al., 1997; Ott et al., 1998). Thus, signaling events at the PSD may regulate the extent of Kv4.2 expression in the surface membrane via its interaction with filamin.

What is the importance of positioning this K⁺ channel in such a restricted manner? The answer may lie not within the ion channel but in the complex with which it is associated. Modulatory enzymes precisely localized to the subsynaptic membrane could provide a rapid activity-dependent mechanism for the regulation of channel expression in the surface membrane and/or channel kinetics, thus modulating postsynaptic excitability. In fact, a role for PKC in Kv4.2 and filamin modulation has been established (Nakamura et al., 1997; Glogauer et al., 1998). Interestingly, PKC has been shown to bind β-integrin (Ng et al., 1999), while the interaction of β-integrin with filamin has been clearly established (Sharma et al., 1995; Loo et al., 1998; Pfaff et al., 1998). Thus, via its interaction with Kv4.2 and β-integrin, filamin may serve as a molecular scaffold to localize Kv4.2 to the postsynaptic membrane and/or to mediate the assembly of a macromolecular complex linking Kv4.2 to the actin cytoskeleton and signaling molecules. A similar signaling complex has been described within which Yotiao, a scaffold protein that directly links the NMDA receptor with type I protein phosphatase and cAMP-dependent protein kinase, facilitates the regulation of channel activity (Westpal et al., 1999).

The ability of filamin to localize Kv4.2 to cellular specializations and stabilize its expression in the surface membrane in heterologous cells identifies it as a candidate protein involved in Kv4.2 localization and surface membrane expression at the synapse. Further characterization of the Kv4.2/filamin interaction in neurons will be required to address the role of filamin in Kv4.2 localization at the neuronal synapse.

**REFERENCES**


