Interaction of SAP97 with Minus-end-directed Actin Motor Myosin VI

IMPLICATIONS FOR AMPA RECEPTOR TRAFFICKING*

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SAP97 is a modular protein composed of three PDZ domains, an SH3 domain, and a guanylate kinase-like domain. It has been implicated functionally in the assembly and structural stability of synaptic junctions as well as in the trafficking, recruitment, and localization of specific ion channels and neurotransmitter receptors. The N terminus of SAP97 (S97N) has been shown to play a key role in the selection of binding partners and the localization of SAP97 at adhesion sites, as well as the clustering of ion channels in heterologous cells. Using the S97N domain as bait in a yeast two-hybrid screen, we identified the minus-end-directed actin-based motor, myosin VI, as an S97N binding partner. Moreover, in light membrane fractions prepared from rat brain, we found that myosin VI and SAP97 form a trimeric complex with the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit, GluR1. These data suggest that SAP97 may serve as a molecular link between GluR1 and the actin-dependent motor protein myosin VI during the dynamic translocation of AMPA receptors to and from the postsynaptic plasma membrane.

The synapse-associated protein SAP97 belongs to the SAP90/PDZ-95 subfamily of membrane-associated guanylate kinase homologs (MAGUKs). This family of proteins contains three PSD95/SAP90-DLG-ZO1 (PDZ) domains, an Src homology 3 domain, and a guanylate kinase-like (GUK) domain. Each domain has been found to be a site of protein-protein interaction (1–3). MAGUKs are found both pre- and postsynaptically within the vertebrate central nervous system, where they are thought to play key roles in the organization of synaptic junctions. More specifically, MAGUKs have been shown to be involved in the targeting of voltage- and ligand-gated ion channels and receptors, anchoring synaptic proteins at the synaptic membrane and organizing signaling complexes (2–8).

Within the nervous system, SAP97 has been found localized within the presynaptic nerve termini of excitatory synapses in the hippocampal formation, at postsynaptic sites within the cerebral cortex, and along bundles of unmyelinated axons in other brain regions (5, 9, 10). In contrast to most other MAGUKs, SAP97 is also expressed in non-neural tissues, where it also exhibits a restricted staining pattern. For example, in epithelial cells, SAP97 and its human homolog, hDlg, have been found localized at sites of cell-cell contact along the lateral membrane where they are integrated into the cortical cytoskeleton (5, 9, 11). This suggests that SAP97 may play a scaffolding role in the assembly and structural integrity of various types of membrane specialization. This concept is supported by studies on Dlg, the SAP97 orthologue in Drosophila, where it is found to be essential for the assembly of septate junctions and epithelial tumor suppression (12, 13) as well as the assembly and function of the larval neuromuscular junction (8). SAP97 can functionally replace Dlg in knock-out flies at the neuromuscular junction and can suppress tumor formation in the imaginal discs (14).

Within the CNS, SAP97 has been implicated in the synaptic localization and membrane trafficking of GluR1, a subunit of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamate receptors (10, 15, 16). Here, GluR1 has been found to interact with the first PDZ domain of SAP97 via its C-terminal tail and co-localize at the postsynaptic density (PSD) in rat cerebral cortex (10, 15). The association of GluR1 and SAP97 appears to occur initially during the early stages of the biosynthesis of GluR1 subunits, as an interaction between these two synaptic proteins has been detected, whereas GluR1 subunits are still in the endoplasmic reticulum and cis-Golgi network (16). SAP97 and GluR1 complexes appear to dissociate as the two proteins reach the synaptic plasma membrane (16). Thus, SAP97 may play a role in the synaptic trafficking of GluR1.

In non-neuronal cell systems, SAP97 appears to be involved in the trafficking of integral membrane proteins to and from the plasma membrane. For example, SAP97 has been found to translocate from a vesicular compartment to sites of cell-cell adhesion in an E-cadherin-dependent manner (11). The analysis of domains essential for the localization of SAP97 at adhesion sites has shown that the N-terminal segment proceeding the PDZ1 domain of SAP97 (S97N) is the most critical (17). Moreover, the S97N region has been found to be a key regulatory element in the selection of SAP97 binding partners including the GUK domain-binding protein, GKAP, and the KA2 subunits of kainate receptors, as well as the formation of homomultimers of hDlg (18–20).
To gain further clues to the function of the S97N domain, we searched for proteins that interact with this domain. Here, we report that the C-terminal cargo binding domain of myosin VI, a minus-end-directed actin-dependent motor, interacts with the N-terminal 104 amino acid residues of SAP97 both in vitro and in vivo. Furthermore, we found that SAP97 and myosin VI form a trimeric complex with GluR1 in vesicular fractions from rat brain. These data suggest that SAP97 may serve as an adapter protein linking myosin VI to vesicular cargoes carrying glutamate receptor subunits.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Goat polyclonal anti-GST antibodies were purchased from Amersham Biosciences. Rabbit polyclonal anti-myosin VI antibody was a generous gift from Dr. Tama Hasson (21). The mouse monoclonal antibodies mAb-77.4 and mAb-119 were produced by the Hybribodies Core Facility at University of Alabama at Birmingham, and rabbit polyclonal antibody 5’31F and 2d have been described previously (5). Both mAb-77.4 and 5’31F were generated against the N-terminal 163 amino acid residues of SAP97 (11). The 2d antibody was raised against the PDZ1–2 domains of SAP90/PSD95. The rabbit polyclonal SAP97 antibody, 62426 III3, was raised against the N-terminal 1–105 amino acid residues of SAP97 (a generous gift from Johannes Tell (10)). The mouse monoclonal antibody mAb-119 was generated against the N-terminal 119 amino acid residues of SAP102 (6). The rabbit polyclonal anti-GluR1 antibody raised against the C terminus of GluR1 subunit was purchased from Chemicon.

**Cell Culture, Transfection, and Immunofluorescence**—The rat adrenal pheochromocytoma PC12 cells were cultured in RPMI 1640 (Invitrogen) media supplemented with 10% horse serum and 5% fetal bovine serum (FBS). The cells were maintained at 37 °C under a 5% CO2 atmosphere.

**Results**

**ELISA—ELISA-based binding assays were performed as described previously (22).** The absorbance at 405 nm was measured in a microplate reader (Cambridge Technology Inc.). The values for each experiment were changed into relative binding activity by defining the maximal absorbance at 405 nm as 100%. All assays were repeated three times in duplicates.

**Overlay Assay**—In overlay assays, protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane. The membrane was blocked with blocking solution (5% skim milk and 0.5% Nonidet P-40 dissolved in Tris-buffered saline (TBS)) followed by incubation with 10 µg/ml His6/7-tagged S97N1-104 or S97N1-119 diluted in 3% FBS/TBS, for 2 h at room temperature. The blots were washed three times with TBS, blocked with blocking solution for 30 min, and incubated with anti-T7 monoclonal antibody for 2 h at room temperature followed by AP-conjugated secondary antibody. The immunoreactive bands were detected after soaking blots in 10 ml of AP staining solution (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl2) containing 165 µg/ml nitro blue tetrazolium (NBT, Sigma) and 82.5 µg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Sigma). The reaction was stopped with distilled water after 5–10 min.

**Pull-down Assay**—Pull-down assays using GST fusion proteins were performed as follows. GST alone or GST fusion proteins were purified as described above and dialyzed for 2 h in PBS using Slide-A-Lyzer (Pierce). Ten µg of GST fusion proteins were then coupled to 50 µl of glutathione-agarose beads for each reaction by incubating them at room temperature for 1 h. After being washed several times with PBS, the beads are ready for the assay.

To prepare cell extracts, confluent cells in 10-cm culture dishes were washed twice with ice-cold PBS. One ml of extraction buffer (1% Triton X-100 in PBS containing 10 µg/ml each aprotinin, leupeptin, and pepstatin and 1 µg/ml phenylmethylsulfonyl fluoride) was then added to each 10-cm dish, and cells were scrapped off with a rubber policeman. After homogenization with a Dounce homogenizer and a 30-min incubation on ice, the samples were centrifuged at 16,000 × g at 4 °C for 30 min. The supernatant (cell extracts) was then incubated with GST fusion protein-coupled glutathione beads for 2 h at 4 °C. The beads were pelleted by centrifugation. After three washes with the extraction buffer and one wash with PBS, 100 µl of SDS sample buffer was used to elute the proteins associated with the glutathione beads. The samples were boiled for 5 min and then processed for SDS-PAGE and Western blotting.

**Immunoprecipitation**—Immunoprecipitation was performed essentially as described previously (23). In brief, cultured PC12 cells in each 10-cm dish were homogenized in radioimmunoprecipitation buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS) and incubated on ice for 30 min to solubilize the cytoskeletal components. After centrifugation at 16,000 × g for 30 min, the supernatant was treated with bovine serum albumin-treated protein A-Sepharose for 1 h at 4 °C, followed by incubation with antibody bound to protein A-Sepharose for 2 h at 4 °C. Immunoprecipitates were washed, separated by SDS-PAGE, and analyzed by Western blotting.

**Preparation of Crude Membrane Fractions**—Crude membrane fractions were prepared from Sprague-Dawley rat brain in brief. In brief, the brains from rats of postnatal day 8 (P8) were rapidly removed after cervical dislocation, homogenized on ice to 1:10 (w/v) homogenization buffer (0.32 M sucrose, 1 mM EDTA, 10 mM Tris (pH 7.4), 0.5 mM phenylmethylsulfonyl fluoride, 2 µg/ml pepstatin A, 2 µg/ml leupeptin). Following centrifugation at 800 × g for 15 min to remove the nuclei and cell debris, the supernatant was centrifuged at 100,000 × g for 30 min at 4 °C. The cleared supernatant (S100) was then retained, and the pellet was resuspended and solubilized for 20 min at 4 °C in Buffer A (150 mM NaCl, 10 mM Tris-HCl pH 7.4, 10 mM EDTA, 10 mM EGTA, 1% Triton X-100). The fractions were boiled in SDS sample buffer for 5 min prior to SDS-PAGE and Western blotting.

**Flotation**—Flotation assays were performed essentially as described previously (28). In brief, P8 brains were dissected and homogenized in homogenization buffer as described above. Homogenate was centrifuged at 800 × g for 15 min to remove nuclei and cell debris. The crude membrane in the supernatant was centrifuged at 100,000 × g for 1 h, lysed hypotonically in 9 volumes of 2 mM Hepes buffer (pH 7.4) for 20 min on ice, and then centrifuged at 100,000 × g for 1 h. The pellet (P100) was adjusted to 2 µl sucrose and loaded as a layer of a discontinuous sucrose gradient underneath layers of 1.2, 0.8, and 0.3 µl sucrose. The gradient was centrifuged at 350,000 × g for 3 h. Fractions were taken from the top to the bottom. Fractions used for immunoprecipitation were resuspended in radioimmunoprecipitation buffer.

**RESULTS**

**S97N Interacts with the C-terminal Region of Myosin VI**—Previous studies have shown that the N-terminal 186 residues of SAP97 (S97N) plays an essential role in the targeting of SAP97 to sites of cell-cell contact, in the multimization of SAP97 molecules and in the selection of binding partners (17–20). Although the latter two interactions are thought to represent both inter- and intramolecular interactions between and within SAP97, the former is conceivably due to intermolecular interactions with other cellular proteins. To gain insight into how the S97N domain functions to direct the localization of SAP97, we performed a yeast two-hybrid-based screen to identify potential S97N binding partners. Three bait libraries, S97N1-104, S97N1-119, and S97N1-126, were used to screen a mouse kidney cDNA library. S97N1-119 was self-activating and was not used further. Several potential binding partners were identified for the S97N1-104 and S97N1-119 baits. Of these, one clone, N104#29, was found to encode the C-terminal region of myosin VI, an actin-based unconventional motor protein (termed myo6-C) (Fig. 1B) (21, 24, 25). This clone is of particular inter-
affinity because actin-based motor proteins are implicated in protein trafficking (26).

To confirm the interaction between S97N and myo6-C, we first performed an ELISA-based binding assay to assess whether these interactions could occur in vitro. In these assays, the myo6-C, as well as two non-interacting clones, N65#23 and N104#8, used as controls, were expressed and purified as GST fusion proteins, and the S97N1–104 and S97N1–104 were fused to the His6/T7 epitope. In each experiment, 20 pmol of His6/T7-tagged S97N1–104 (Fig. 1C) or S97N1–104 (Fig. 1D) were bound to a 96-well ELISA plate and incubated with increasing concentrations of GST or GST-tagged N65#23, N104#8, and myo6-C. The binding activity was detected by anti-GST antibody, followed by AP-conjugated secondary antibody. Myo6-C was found to bind both S97N fragments, although binding was stronger with S97N1–104 than S97N1–104. Clones N65#23 and N104#8 were unable to interact with S97N1–104 or S97N1–104 (Fig. 1, C and D).

As a second measure that the S97N domain can interact with the C-terminal tail of myosin VI, we performed an overlay assay. Here, GST and GST-tagged myo6-C were immobilized on nitrocellulose membrane, incubated with 10 μg/ml His6/T7-tagged S97N1–104 or S97N1–104, and then stained with anti-T7 antibody. Again, both S97N1–104 and S97N1–104 were found to interact with GST-tagged myo6-C (Fig. 2A). As observed in our ELISA, S97N1–104 appeared to bind more strongly to myo6-C than S97N1–104, as assessed by the intensity of the bands (Fig. 2A). These data indicate that most of the first 104 amino acid residues S97N1–104 are required for strong binding to this C-terminal segment of myosin VI.

Affinity Purification of SAP97 and Myosin VI—Our initial in vitro binding data demonstrated that short regions of SAP97 (S97N1–104) and myosin VI (myo6-C) can interact with each other (Figs. 1 and 2A). To determine whether these regions could interact when placed in the context of full-length molecules, a GST-based “pull-down” assay was utilized (Fig. 2, B and C). PC12 cell extracts were used as the source for full-length SAP97 and myosin VI, because both proteins are endogenously expressed in these cells. When GST-tagged S97N1–104 was immobilized on glutathione-agarose beads and incubated with total cell extracts of PC12, endogenous myosin VI was found to bind the GST-S97N1–104 beads but not the control GST beads (Fig. 2B). Similarly, incubating PC12 cell extracts with GST-my6-C-bound glutathione-agarose beads lead to the specific isolation of SAP97 (Fig. 2C). These data suggest that S97N1–104 and myo6-C interact in the context of full-length molecules.

Although members of the SAP97 family share a high degree of homology between their PDZ, Src homology 3, and GUK domains, little if any similarity exists between their N-terminal sequences (17). To further explore the specificity of the myosin VI-SAP97 interaction, we examined whether GST-myo6-C could affinity purify another member of the SAP97 family. This was accomplished by transfecting myc-tagged SAP102 and SAP97 into COS7 cells and subjecting the cell extracts to pull-down with GST-myo6-C. Although myc-tagged SAP97 was readily detected in the bound fraction of GST-myo6-C, in three separate experiments myc-tagged SAP102 was not detected (Fig. 2D), indicating that the interaction between myosin VI with SAP97 is specific.
To assess the importance of the interaction between SAP97 and myosin VI, we compared the distribution pattern of SAP97 and myosin VI in PC12 cells by double-labeling immunofluorescence microscopy. In undifferentiated PC12 cells, e.g. those that had not been treated with nerve growth factor (NGF), SAP97 exhibited a punctate cytoplasmic pattern as well as a pronounced immunoreactivity at sites of cell-cell contact (Fig. 3a). Myosin VI also exhibits a punctate cytoplasmic pattern similar to SAP97, and yet it was not found at cell-cell adhesion sites (Fig. 3b). The punctate pattern observed for myosin VI is similar to that observed previously for myosin VI in neuronal cells (27). Given the small size of these undifferentiated cells, it was not feasible to determine accurately whether SAP97 and myosin VI truly co-localize. We therefore treated PC12 cells for 3 days with NGF. This treatment induced the outgrowth of neurites as well as the loss of cell-cell adhesion sites (Fig. 3c). In this situation, cells double-labeled with SAP97 and myosin VI antibodies revealed a partially overlapping punctate pattern for these two proteins (Fig. 3, d–f). This suggests that both myosin VI and SAP97 are present on a population of vesicles within PC12 cells. The fact that there is only a partial overlap is somewhat expected given that SAP97 is thought to move with its ion channels via vesicular membranes both to and from the plasma membranes (11), whereas myosin VI is thought to be involved primarily in the retrieval of membrane proteins from the plasma membrane (26).

Further support that SAP97 and myosin VI physically interact with each other in these cells was obtained by immunoprecipitating SAP97 with either our 5′31f or 2d antibodies from cellular extracts from both NGF differentiated and undifferentiated cells. The 5′31f antibody recognizes the S97N domain of SAP97, and the 2d antibody recognizes the PDZ1–2 domain in SAP97 (5). Probing Western blots of these immunoprecipitates with antibodies against SAP97 and myosin VI showed that both were found in the precipitates with anti-SAP97 antibodies prepared from both NGF-treated and untreated cells but not in precipitates with a control IgG (Fig. 4). These data suggest that SAP97 and myosin VI can interact in PC12 cells, indeed on a subpopulation of vesicles.

**SAP97 and Myosin VI Do Not Interact in Colonic Epithelial Cells**—In non-neuronal cells, SAP97 has been found to exist as a soluble protein in association with vesicular membranes, and as a structural component of the cortical cytoskeleton assembled at sites of cell-cell contact (11). The latter is readily observed in undifferentiated PC12 cells (Fig. 3) as well as columnar epithelial cells such as CACO2 or MDCK (11). Similarly, myosin VI can exist as a soluble protein, in association with vesicular membranes, and at least in the inner ear is associated with the actin-rich microvilli of the hair cell epithelium (21). These observations suggest that the myosin VI and SAP97 interaction could potentially occur in a variety of situations, e.g. as soluble proteins, with vesicular membrane as suggested from our PC12 experiment, and/or in association with the cortical cytoskeleton. Our previous studies on the colonic epithelial cell line, CACO2, show that nearly all of the SAP97 is found at cell-cell adhesion sites, with very little in the cytoplasm (11). We therefore examined this system to assess whether myosin VI interacts with SAP97 when it is assembled into the cortical cytoskeleton. Although both proteins were found to be expressed in these cells (Fig. 5A), they exhibit distinct distribution patterns as assessed by double-label immunofluorescence microscopy (Fig. 5C). For example, SAP97 is found at adhesion sites, whereas myosin VI exhibits a punctate cytoplasmic distribution. Furthermore, immunoprecipitating SAP97 with our 5′31f antibody failed to detect any myosin VI in the complex (Fig. 5B). These observations indicate that SAP97 and myosin VI most likely interact in the cytosol either as soluble proteins or in association with vesicular membrane rather than at cell-cell adhesion sites and are consistent with our data on PC12 cells.
**SAP97 and Myosin VI Interact in PC12 Cells**

Myosin VI was co-immunoprecipitated with SAP97, indicating that myosin VI does not interact with SAP97 in IgG precipitates. Although initially confusing, this combination of results is predicted based on our mapping of the epitopes recognized by 62426III3 and 531f (residues 1–50 and 1–163 of SAP97, respectively) and the myosin VI binding site on SAP97 (residues 1–104) (Fig. 1A). As such, whereas 62426III3 is expected to immunoprecipitate SAP97, this antibody, because of steric hindrance, is not expected to have access to its epitope when myosin VI is bound at its predicted binding site on SAP97. SAP97, Myosin VI, and GluR1 Form a Complex in Rat Brain—Given that myosin VI is predicted to be actin motor involved in vesicular membrane trafficking, we also wanted to examine whether SAP97 and myosin VI proteins interact within the context of a vesicular cargo. Recent studies by ourselves and others (15–16) have discovered that the GluR1 subunit of the AMPA receptors is a naturally occurring binding partner for SAP97 and that SAP97 may have a role in the trafficking of these receptors (16). A possible association of these three proteins on vesicular membranes was initially investigated by comparing the buoyant properties of myosin VI, SAP97, and GluR1 using a flotation assay that we had used previously to show that the presynaptic active zone proteins Piccolo and Bassoon are transported to nascent synapses in association with an 80-nm precursor vesicle (28). In this assay, P100 membrane fractions prepared from P8 rat brain were adjusted to 2M sucrose and loaded at the bottom of a discontinuous sucrose gradient. After centrifugation, 0.5-ml fractions

**FIG. 4. Interactions of SAP97 with myosin VI in PC12 cells.** A, Western blots of SAP97 and myosin VI from PC12 cells (not treated with NGF) immunoprecipitated with antibodies against SAP97 (531f) or irrelevant IgG. The precipitates were blotted with a SAP97 monoclonal antibody 77.4 and myosin VI polyclonal antibody. The input is about 1/50 of total cell extracts used in the immunoprecipitation assay. B, confirmation that SAP97 can interact with myosin VI in PC12 cells, cell extracts were subjected to immunoprecipitation (IP) with a second SAP97 antibody, 2d, that recognizes the PDZ1–2 domain region of SAP97. In both NGF-treated (+NGF) and untreated (–NGF) extracts, but not in IgG precipitates, myosin VI was co-immunoprecipitated with SAP97. 531f, precipitate.

**FIG. 5. Expression and distribution of SAP97 and myosin VI in CACO-2 cells.** A, Western blots of CACO-2 cell extracts incubated with a rabbit anti-myosin VI polyclonal antibody (lane 1) or the anti-SAP97 monoclonal antibody 77.4 (lane 2). B, Western blots of CACO-2 cell extracts subjected to immunoprecipitation with a control IgG or antibodies (531f) against SAP97. Myosin VI was not found in these precipitates, indicating that myosin VI does not interact with SAP97 in CACO-2 cells. C, immunofluorescent micrographs of CACO-2 cells double-labeled with mouse antibodies against SAP97 (77.4) (green) and rabbit polyclonal antibodies against myosin VI (red). SAP97 (a) is restricted to the lateral membrane, and myosin VI (b) is present in the cytoplasm exhibiting a dense punctate pattern.
DISCUSSION

In this study, we discovered that the multi-domain adaptor protein SAP97 can interact with an unconventional actin-based motor protein, myosin VI. The interaction appears to occur not only in vitro but also in vivo. Our studies using rat brain extracts suggest that SAP97 can interact with myosin VI in light microsomal membrane fractions and that this complex can contain GluR1 subunits of AMPA-type glutamate receptors.

Unconventional or non-muscle-type myosins are involved in many cellular processes such as cell growth and development, cell movement, RNA transport, organelle transport, particle movement, endocytosis, and exocytosis (26, 29, 30). At present, the biological importance of the interaction between SAP97 and myosin VI is unclear. As an actin-based motor protein, myosin VI could be involved in protein/membrane trafficking of SAP97 and its associated proteins. Alternatively, it may function as an adaptor protein to anchor SAP97 to the actin cytoskeleton, thus playing a role in the subcellular targeting of SAP97 to its membrane specialization, such as the cortical cytoskeleton along the lateral membrane of epithelial cells. Although this may be possible in specialized cell types, such as those found in the cochlear, our initial studies on a colonic epithelial cell line, CACO-2, indicate that this is not always the case, as SAP97 and myosin VI were found to exhibit distinct distribution patterns in these cells and could not be detected in a protein complex as assessed by immunoprecipitation.

A more likely scenario is one wherein myosin VI is involved in the trafficking of vesicle-associated SAP97. This concept is supported by several distinct kinds of data. First, we found that SAP97 and myosin VI exhibited a punctate overlapping pattern in the cytoplasm of differentiated and undifferentiated PC12 cells. As in CACO2 cells, myosin VI did not co-localize with SAP97 at sites of cell-cell contact, suggesting that SAP97 and myosin VI interact, as detected by immunoprecipitation in extracts from these cells is occurring on vesicular membranes. This hypothesis is supported by our biochemical analysis of the association of SAP97 and myosin VI in rat brain homogenates. Here, using a flotation assay, it was possible to show that both SAP97 and myosin VI can be found associated with light membranes, as assessed by immunoprecipitation. Although it was not shown directly, these findings suggest that myosin VI could be the motor protein that moves the vesicles containing SAP97. Given the fact that the SAP97-interacting domain on myosin VI is its C-terminal cargo binding domain, we hypothesize that SAP97 could serve as an adapter protein linking this motor to its vesicular cargo.

An important unresolved question is under what conditions myosin VI and SAP97 interact, and whether myosin VI participates in trafficking SAP97-associated vesicular membranes or from the plasma membrane. Myosin VI is a minus-end-directed, actin-dependent motor protein that is thought to move the particles/vesicles toward the minus or pointed end of
actin filaments (25). Thus, knowledge of the polarity of cellular actin filaments will be necessary for understanding the potential functions of myosin VI (30–31). The best characterized site of actin filaments nucleation is the plasma membrane where their barbed (+) ends are attached to the plasma membrane and the pointed (−) ends are buried in the cell body (32–36). In addition, networks of cortical actin filaments, some of which remain membrane-attached, are most often of mixed polarity (37). Therefore, vesicles carried on these actin filaments by myosin VI are expected to move away from or along the plasma membrane. Thus, myosin VI is likely to facilitate the retrieval of SAP97-associated vesicles from the plasma membrane, as occurs during the loss of calcium/C-adenosin-dependent cell-adhesion (11). This hypothesis is consistent with a recent study demonstrating that a splice variant of myosin VI is localized to clathrin-coated vesicles and can modulate clathrin-mediated endocytosis (38).

Interestingly, GluR1, a subunit of AMPA-type glutamate receptors shown to interact with SAP97 (15–16), was found to form a complex with SAP97 and myosin VI in rat brain. Although this was not tested, SAP97 is predicted to be the molecular link between GluR1 and myosin VI, given that SAP97 can interact directly with both GluR1 (via its first PDZ domain (15)) and myosin VI (via its S9T7N domain) (Fig. 1). Complex formation between these three proteins appears to occur on vesicular membranes, as the three proteins could be co-immunoprecipitated from the same light membrane fraction. An appealing hypothesis is that GluR1-SAP97-myosin VI complex forms an intracellular trafficking particle wherein myosin VI is the motor protein that moves the particle. This scenario is particularly interesting in the postsynaptic domain of a glutamatergic synapse where AMPA receptors continuously recycle between the plasma membrane and the intracellular compartments (39–46). The recycling of AMPA receptors is thought to occur via clathrin-dependent endocytosis and vesicle-mediated membrane insertion (39, 40, 46). Given that dendritic spines are enriched in actin filaments, an actin-based transport system is likely to be responsible for the local recycling of AMPA receptors in the postsynaptic plasma membrane. As an actin-based, minus-end-directed motor protein, myosin VI is predicted to participate in the endocytosis process of AMPA receptors. This is supported by the discovery that myosin VI is indeed associated with clathrin-coated vesicles (38). However, contradictory to this hypothesis, a recent study has shown that the interaction of SAP97 and GluR1 occurs primarily early in the secretory pathway when receptors are in route to the plasma membrane from the endoplasmic reticulum and cis-Golgi (16). A role for SAP97 in the internalization of GluR1 has not been well investigated and will require additional evidence before a clear role for myosin VI in the recycling of AMPA receptors can be elucidated.

Intriguingly, myosin VI is not the only motor protein found to form a complex with members of the SAP97 family of MAGUKs. In fact, myosin V, a plus-end-directed actin motor, was recently found to form ternary complex with SAP90/PDS95 via GAKAP and dynein light chain (47). SAP97 also interacts with GAKAP in the nervous system via its GUK domain (18, 22) and thus is also expected to form a complex with myosin V. Given the directional property of myosin V and myosin VI, it seems reasonable to hypothesize that myosins V and VI function in concert, regulating the movement of SAP97-containing vesicles along actin filaments. Finally, the human homolog of SAP97, hDlg, recently has been found to interact with a microtubule-based kinesin-like motor protein, GAKIN, via its GUK domain (48). Whether SAP97 can bind GAKIN and GAKAP at the same time is not known. Nonetheless the ability of SAP97/hDlg to interact with both actin and microtubule-based transport systems places this multidomain adapter proteins in the center of organizing and directing the trafficking of vesicle-associated protein complexes in a variety of cell types.
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