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THE POSTSYNAPTIC DENSITY PROTEIN ABELSON INTERACTOR PROTEIN 1 INTERACTS WITH THE MOTOR PROTEIN KINESIN FAMILY MEMBER 26B IN HIPPOCAMPAL NEURONS

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Abstract—Abelson interactor protein 1 (Abi-1) localizes to postsynaptic densities (PSDs) of excitatory synapses and was shown to be transported from the PSD to the nucleus and back depending upon synaptic activation. We employed a yeast-two-hybrid screen to search for putative transport molecules. We found Kif26B a member of the Kif family of transport proteins that has not been characterized in the central nervous system as a direct interaction partner of Abi-1. We delineated a proline-rich motif within the cargo-binding domain of Kif26B to be responsible for this protein–protein interaction. Kif26B was able to recruit Abi-1 to the microtubule network and we found that the expression of Kif26B is responsible for the localization of Abi-1 to PSDs in maturing neurons. Taken together we report that Abi-1 is a cargo of Kif26B in primary hippocampal neurons, pointing to a role of this transport molecule in the movement of Abi-1 between different cell compartments. Additionally, we provide the first detailed investigation of Kif26B and its cargo molecules in neuronal cells.

Keywords: neuron, motor proteins, PSD, Abi-1, Kif26B, microtubules.

INTRODUCTION

The Abelson interactor protein 1 (Abi-1) is a multidomain protein involved in a variety of cellular mechanisms such as cell proliferation, tumor progression, cytoskeletal rearrangement or has been shown to act within different signaling cascades (Roffers-Agarwal et al., 2005; Proepper et al., 2007; Cui et al., 2009; Chen et al., 2010). In the nervous system it is localized to the postsynaptic density (PSD), the neuronal compartment which receives and transduces incoming signals from other nerve cells. Abi-1 is a key regulator of various macromolecular complexes that influence local cytoskeletal dynamics and modulate synaptic development. In response to synaptic activation Abi-1 was found to be transported to the nucleus where it might act as a co-factor in transcriptional regulation (Proepper et al., 2007). In general, intracellular transport is realized via transport proteins or transport complexes which are able to actively translocate along the actin cytoskeleton or filamentous microtubular structures of neurons (Cai and Sheng, 2009). In neurons the transport is of particular importance, since the cargo has to be transported over long distances in both directions (anterograde and retrograde) and needs to be selectively moved throughout the different neuronal compartments, such as the dendrites and the axon (Hirokawa and Takemura, 2004). In the developing neuron the directed and specific transport of e.g. preformed synaptic protein complexes or organelles to various compartments are of major importance. Several different classes of motor proteins have been shown to be involved in these highly elaborated transport events. While Kif proteins and the Dynein/Dynactin motor complex are responsible for the transport along microtubules, proteins of the myosin family make use of actin filaments (Vale et al., 1985; Lise et al., 2006; Zheng et al., 2008). Microtubules and actin filaments are the major cytoskeletal structures that stabilize cell morphology, and cross through the cell cytoplasm as long macromolecular filaments (Kapitein and Hoogenraad, 2011). Microtubule formation and degradation in the cell is a complex mechanism that involves e.g. capping structures to define the growing or shrinking end of the microtubule (Kapitein and Hoogenraad, 2011). In neurons microtubules are specifically oriented with the plus end only reaching outward in the axon and in peripheral dendrites, where they even reach the base of dendritic spines (Hu et al., 2008; Kapitein et al., 2010). In the main dendritic tree, microtubules show mixed polarity. It has been shown already that the further extension of microtubules throughout the dendritic spine, a structure that is crucial for synaptic development and plasticity, is synapse activity dependent (Jaworski et al., 2009; Kapitein et al., 2010). In the most outward regions of a synaptic contact, however, such as the presynaptic terminal or

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Abbreviations: Abi-1, Abelson interactor protein 1; CA1, Cornu Ammonis 1; Cos-7, Cercopithecus aethiops, origin-defective SV-40 cell line 7; DAPI, 4,6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; GAL4, Galactosidase transcription activator protein 4; GFP, green fluorescent protein; Kif, Kinesin protein family; Kif26B, Kinesin family member 26B; MAP2, microtubule associated protein 2; Myc, myelocytomatosis oncogene; PBS, phosphate-buffered saline; PD, proline-rich synapse-associated protein-1; ProSAP1, pull down experiments; ProSAP1, proline-rich synapse-associated protein-1; PSDs, postsynaptic densities; RT, room temperature; SHANK, SH3 and multiple ankyrin repeat domains protein.

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the postsynaptic spine end, myosins are responsible for transport of cargos as these structures lack microtubules.

The Kinesin protein family (Kif) consists of 45 known members that carry cargo along the microtubule network in an ATP-dependent manner (Miki et al., 2001). Kif proteins harbor a so-called cargo-binding domain that is able to attach molecules that are meant to be transported between the different cell compartments. Transported cargos are among others, organelles, protein complexes and RNA molecules. Additionally, Kif proteins have been demonstrated to be involved in the formation and function of the spindle apparatus during cell division (Hirokawa et al., 1998). The Kinesin family member 26B (Kif26B) has up to now solely been reported to be differentially expressed during embryonic development of somites or kidney mesenchyme in early stages of embryonic development (Marikawa et al., 2004; Uchiyama et al., 2010). Recent findings document the expression of Kif26B in the nervous system and protein expression was found to be altered during pathological processes (Hashimoto et al., 2011). The present study reveals the abundance of Kif26B in the nervous system at later developmental stages and its functional protein–protein interaction with Abi-1. Kif26B is thereby able to modulate the presence of Abi-1 in the synaptic compartment. These data therefore point to a role of Kif26B in the transport of Abi-1 to different cellular compartments, especially to the PSD of excitatory synapses.

EXPERIMENTAL PROCEDURES

Ethics statement

All animal experiments were performed in compliance with the guidelines for the welfare of experimental animals issued by the Federal Government of Germany, the National Institutes of Health and the Max Planck Society. The experiments in this study were approved by the review board of the Land Baden Württemberg, Permit Number Nr. O.103.

Yeast-two-hybrid screen

A yeast-two-hybrid screen was performed essentially as described previously (Proepper et al., 2007; Liebau et al., 2009). In brief, *Saccharomyces cerevisiae* yeast strains were used and screening was based on the generation of a functional Galactosidase transcription activator protein 4 (GAL4) transcription factor that drives the specific lacZ reporter gene. As bait full-size Abi-1 was fused to the GAL4 DNA-binding domain in vector pGBKT (Clontech, San Diego, CA) screened against a human fetal brain library cloned into the pACT2 vector (Clontech). Following overnight cultivations of the bait in SD-Trp at 200 rpm and 30 °C, mating between the two was performed in YPD medium overnight at 40 rpm and 30 °C. Mated cells were harvested at 1000*g* for 10 min and resuspended in 20-ml YPD. This solution was plated on close to 50–150 mm selection plates (SD-His, -Leu, -Trp + 3-AT). Colonies positive for interaction were detected by a blue coloration after being subjected to a Filter Lift Assay (FLA). DNA from blue colonies was extracted and interacting partners were identified by sequencing such as the c-terminal part of human Kif26B (GI:142370197).

In situ hybridization

In situ hybridization was performed according to Liebau et al. (2011a). In brief, radioactively labeled cDNA probe was added to 60 µl of salmon testes DNA and incubated for 5 min at 90 °C followed by 3 min on ice. Six hundred microliters of hybridization cocktail and 30 µl of tRNA were added. Seventy microliters of the hybridization solution was pipetted onto each section avoiding bubbles and incubated overnight at 42 °C. Sections were washed four times for 15 min with 1× SSC/0.1% β-mercaptoethanol and two times with 0.5× SSC/0.1% β-mercaptoethanol at 58 °C. Samples were cooled down in wash buffer for 15 min at RT, followed by washing three times with 1× SSC, one time with 0.5× SSC and 0.25× SSC at RT, 5 min each. Dehydration of the sections was performed at RT transferring the sections to increasing grades of ethanol for 15 s and ethanol abs. for 15 s. The dried sections were then placed into an X-ray film cassette and transferred to a dark room. Exposure of the sections to an X-ray film took place for 8–24 days before the film was finally developed. To detect Kif26B transcripts in rat brain sections we used a S^{35}-labeled cDNA antisense oligonucleotide (5′-CTG CTA TCC AGT TCT TGC TCT GAC TTA CCT TC-3′, bp 4435–4466) from MWG-Biotech (Ebersberg, Germany).

Cell culture

Preparation of primary hippocampal neurons from rat embryos was adopted from Proepper et al. (2007) and Grabrucker et al. (2011a,b). In brief, embryos (embryonic stage E18/E19) from Sprague Dawley rats (Crl:CD; Charles River, Wilmington, MA) were removed from the uterus. After dissection, the embryonic hippocampi were dissociated with 0.25% trypsin in HBSS (PAA, Pasching, Austria), followed by treatment with 0.01% DNase I (Invitrogen, Darmstadt, Germany). Cells were plated at a density of 30 000 cells/cm^2 on poly-L-lysine-treated glass coverslips in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS). After 3 h, medium was changed to Neurobasal (Invitrogen) supplemented with B-27, 0.5 mM L-glutamine, and 100 units/ml penicillin/streptomycin (all from Invitrogen). The cells were cultured in a humidified incubator at 37 °C and 5% CO_2. Cercopithecus aethiops, origin-defective SV-40 cell line 7 (Cos-7) cells (obtained from DSMZ, Braunschweig, Germany) were cultured in DMEM with high glucose (Invitrogen, Karlsruhe, Germany) and complemented with 10% (v/v) FCS. Cells were grown on commercially available chamber-slides (Nunc, Wiesbaden, Germany) coated with poly-L-lysine (0.1 mg/ml; Sigma–Aldrich, Steinheim, Germany).

Overexpression constructs and transfection

The pEGFP (C1–3) vector system (Clontech) as well as pCMV-Myc (Clontech) were used to clone full-size mouse Kif26B (kind gift of Ryuichi, Nishinakamura, Japan, GenBank ID: NM_001161665.1, 6339 bp), Kif26B MD (motor domain, 1529 bp), Kif26B CBD (cargo-binding domain, 1819 bp) as well as Kif26B CBDmut (mutated cargo-binding domain, 1819 bp), respectively. The mutated cargo-binding domain of Kif26B (Kif26B CBDmut) was generated by using the QuickChange®II Site-Directed Mutagenesis kit according to the manufacturer (Stratagene, San Diego, CA, USA). The rat Abi-1 construct (476 bp) was described previously (Proepper et al., 2007). Transfection experiments in Cos-7 cells were performed using the transfection reagent PolyFect® (Qiagen) according to the manufacturer's recommendations. Additionally Cos-7 cells transfected with recombinant Kif26B-Myc and Abi-1-GFP were treated with 10 µM colchicine (Sigma–Aldrich) for 1 h to disrupt the microtubule system. Hippocampal neurons were transfected with Optifect (Invitrogen) according to the company's instructions. Respective constructs were overexpressed for 24 h in culture.

Immunoprecipitation and pull down experiments

Protein of cultured hippocampal neurons DIV21 was extracted with Triton X-100 lysis buffer (Miltenyi Biotech, vantaa Finnland) and incubated for 2 h at 4 °C by shaking. The immunoprecipitation