Gas7b (Growth Arrest Specific Protein 7b) Regulates Neuronal Cell Morphology by Enhancing Microtubule and Actin Filament Assembly*

Aina Gotoh, Masafumi Hidaka, Keiko Hirose, and Takafumi Uchida

From the Molecular Enzymology, Department of Molecular Cell Science, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi 981-8555, Japan and the Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology, 1-1-1 Higashi, Tsukuba 305-8561, Japan

**Background:** Growth arrest specific protein 7b (Gas7b) protein levels are significantly reduced in the brain tissue of patients with Alzheimer disease.

**Results:** Gas7b enhances microtubule bundling and microtubule cross-linking with the actin filament.

**Conclusion:** Gas7b regulates neural cell morphology by altering the cytoskeletal organization.

**Significance:** A decrease in Gas7b levels impairs neural cell function.

Neurons undergo several morphological changes as a part of normal neuron maturation process. Alzheimer disease is associated with increased neuroproliferation and impaired neuronal maturation. In this study, we demonstrated that Gas7b (growth arrest specific protein 7b) expression in a neuronal cell line, Neuro 2A, induces cell maturation by facilitating formation of dendrite-like processes and/or filopodia projections and that Gas7b co-localizes with neurite microtubules. Molecular analysis was performed to evaluate whether Gas7b associates with actin filaments and microtubules, and the data revealed two novel roles of Gas7b in neurite outgrowth: we showed that Gas7b enhances bundling of several microtubule filaments and connects microtubules with actin filaments. These results suggest that Gas7b governs neural cell morphogenesis by enhancing the coordination between actin filaments and microtubules. We conclude that lower neuronal Gas7b levels may impact Alzheimer disease progression.

Neurogenesis plays an important role in maintenance of memory and learning (1). Neurons undergo morphological changes during their development. These changes have been characterized in hippocampus neurons and Purkinje cells and are initiated by neurite outgrowth, followed by axonal and dendritic branch formation (2, 3). Studies that characterize mature neurons in various brain areas (such as the hippocampus region) of Alzheimer disease (AD)² patients show that there is impaired neuron maturation (4, 5) with increased neuron proliferation (6). Additionally, treatments that promote neuron maturation may improve cognition in AD patients (7).

Microtubules (MTs) and actin filaments (F-actin) are types of cytoskeletal filaments that determine the cell morphology and regulate various cellular activities such as cell division, cell differentiation, cell motility, and intercellular transport (8). The structure and function of cytoskeletal filaments are regulated by many proteins such as microtubule-associated proteins, N-WASP, and fascin. The cytoskeletal filament regulatory proteins are therapeutic targets in diseases such as cancer and AD (9).

Gas7b (growth arrest-specific protein 7b) is primarily expressed in differentiated brain cells such as cortex neurons, hippocampus neurons, and cerebellar Purkinje cells (10). Previous studies indicate that Gas7b alters cellular morphology (10, 11) and that neurite outgrowth was a major phenotypic characteristic of Neuro 2A cells transfected with Gas7b (12). We have demonstrated in a previous study that Gas7b protein levels are lower in brain tissue of AD patients than those in the brain tissue of normal individuals and that Gas7b is involved in neuronal development (13). These findings show that Gas7b is possibly involved in the development and/or progression of AD.

Gas7b consists of a WW domain and an F-BAR (Bin-Amphiphsyn-Rvs) domain. The WW domain of Gas7b shows significant functional similarity with that of Pin1, and the WW domains of both proteins can bind to phosphorylated Tau (13, 14). The BAR domain is a highly conserved domain that influences cell membrane dynamics (15). Several members of the BAR domain super family provide a link between the membrane and the membrane-associated cytoskeleton and play essential roles in fundamental biological processes such as endocytosis, exocytosis, and cell motility (16, 17). Gas7b binds actin via its F-BAR domain to enhance actin polymerization (18). Recently, we demonstrated that excess Gas7b protein directly binds to microtubules and inhibits the motility of kinesin (19).

In this paper, we report two novel functions of Gas7b in neuronal cells. We showed that oligomeric Gas7b enhances (i) bun-
Gas7b Assembles Microtubule and Actin Filament

EXPERIMENTAL PROCEDURES

Western Blot Analysis—Western blot analysis was performed to detect Tau protein in cultured cells by using antibody against Tau 5 (BD Biosciences) as described in our previous report (13).

Characterization of Cytoskeleton and Gas7b Expression in Neuro 2A Cells—GFP and GFP-Gas7b expression vectors were constructed as described in a previous study (13). Nocodazole (NOC), a MT polymerization inhibitor, was diluted in dimethyl sulfoxide. Neuro 2A cells were cultured in DMEM supplemented with 10% fetal bovine serum. The cells were transfected with expression vectors encoding GFP or GFP-Gas7b by using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer’s protocol. After 48 h, the cells were incubated in DMEM with 5 µM NOC for 30 min at 37 °C followed by treatment with 4% of paraformaldehyde for cell fixation. The fixed cells were stained with a monoclonal anti-α-tubulin antibody (Sigma-Aldrich) and an Alexa 594-labeled secondary antibody. The nuclei were stained with Hoechst 33342. The stained cells were observed by using a fluorescence microscope (Biozero 8100; Keyence).

Figure 1. Effect of Gas7b on cell shapes of Neuro 2A. Predominant shapes of Neuro 2A transfected with GFP or GFP-Gas7b are shown. NOC treatment was performed by incubation of the cells with 5 µM NOC for 30 min. A, the cells were fixed and stained with monoclonal anti-α-tubulin antibody (Sigma) and then with Alexa 594-labeled secondary antibody. Green, GFP or GFP-Gas7b; red, α-tubulin; blue, nucleus. Scale bars, 50 µm. B, Neuro 2A cells were classified into three groups: none indicates granule cells without neurites; slender indicates the cells with slender neurites; and irregular indicates the cells with dendrite-like branched processes. ● indicates granule cells without neurites. ● indicates slender neurites. * indicates dendrite-like neurites in irregular shape. C, populations of type of Neuro 2A cell shapes. The cells (n = 80) were classified into three groups. D, Neuro 2A cells were harvested and sonicated in SDS sample buffer and subjected to Western blot analysis with Tau5 antibody. HT4 and − indicate Neuro 2A cells transfected with and without human Tau, respectively.
Protein Preparation—His-tagged Gas7b protein and His-tagged Gas7b variants (Gas7a and F-BAR domain of Gas7b) were expressed in Escherichia coli (BL21) and purified as described in a previous study (20). Gas7b variants were suspended in a buffer appropriate for each assay (polymerization assay, binding assay, and size exclusion assay) and then used for dialysis. MT-associated protein-free tubulin was obtained from bovine brains and purified by using a high molarity buffer protocol (21). Actin was purified as described previously (22).

Microtubule and F-actin Polymerization—MTs at various concentrations (specified in the figure legends) were polymerized in PM buffer (100 mM PIPES, pH 6.9, 5 mM EGTA, 2 mM MgCl₂, 1 mM GTP) at 37 °C. F-actin was polymerized in F buffer (10 mM Tris-HCl, pH 7.5, 1 mM MgCl₂, 50 mM KCl, 1 mM EGTA, 0.2 mM ATP) at room temperature.

Turbidity-based Assay—MT polymerization was analyzed by using a turbidity-based assay (23), which measured the optical density of the polymerized MT solution at 350 nm (∆OD₃50). Turbidity of bundled MTs was also evaluated as follows. Polymerized MTs were incubated with 80 μM of paclitaxel (PTX) for 10 min in PM buffer and then incubated with 80 μM of PTX for 10 min. Turbidity of the mixture of the PTX-MTs (1.0 mg/ml) and Gas7b (0–4 μM Gas7b) with 10 μM NOC or 10 μM of PTX were measured at 37 °C.

FIGURE 2. Effects of Gas7b on MT polymerization and bundling. A and B, MT polymerization assay based on turbidity (A) and fluorescence (B). Tubulin (1.68 mg/ml) was incubated at 37 °C. C, co-precipitation assay of tubulin and Gas7b under the same condition of turbidity assay. The amounts of polymerized MT (Pre.), unpolymerized tubulin (Sup.), and Gas7b were quantified by band densitometry of Coomassie Brilliant Blue-stained SDS-PAGE. D, turbidity based bundling assay of prepolymerized paclitaxel-MTs (PTX-MTs) in the presence of Gas7b. Tubulin (4.0 mg/ml) was polymerized at 37 °C for 10 min in PM buffer and then incubated with 80 μM of PTX for 10 min. Turbidity of the mixture of the PTX-MTs (1.0 mg/ml) and Gas7b (0–4 μM Gas7b) with 10 μM NOC or 10 μM of PTX were measured at 37 °C.
Gas7b Assembles Microtubule and Actin Filament

Gas7b (final concentration, 4 or 6 μM), the A_{250} was measured continuously.

Fluorescence-based Assay—MT polymerization was analyzed by using fluorescence-based methods using DAPI as described previously (24, 25). The fluorescence intensity of MT suspended in PM buffer containing 5 μM DAPI was measured by using Infinite F200 (TECAN). The excitation and emission wavelengths were 360 and 465 nm, respectively.

Co-precipitation Assay—MTs were polymerized in PM buffer at 37 °C for 60 min. The polymerized MTs and unpolymerized tubulins were separated as precipitates and supernatant, respectively, by using high speed centrifugation (20,000 × g for 20 min). Precipitates were resuspended in PM buffer and used to perform SDS-PAGE followed by Coomassie Brilliant Blue staining.

Pulldown Assay—Tubulin (170 pmol), monomeric globular actin (G-actin, 170 pmol), His-tagged Gas7b, His-tagged Gas7b variants (210 pmol), and Ni-NTA resin (Qiagen) were suspended in 50 μl of PM buffer and mixed gently at 4 °C for 1 h. Proteins that interacted with immobilized Gas7b and its variants were bound to the Ni-NTA resin and were precipitated by high speed centrifugation (20,000 × g for 5 min). After performing washes with PM buffer containing 10 mM imidazole, proteins were eluted by addition of PM buffer containing 1 M imidazole. Elutions were analyzed by SDS-PAGE, and the separated proteins were Coomassie Brilliant Blue-stained.

Dark Field and Electron Microscopy Analyses—MTs were analyzed with a dark field microscope BX51 (Olympus) and an EM as described previously (20). To evaluate the MT bundling effect of Gas7b, PTX-MTs with or without Gas7b were negatively stained and visualized by using an EM. To analyze Gas7b-mediated cross-linking of PTX-MTs and F-actin, Gas7b was incubated with PTX-MTs and F-actin in F buffer and then negatively stained followed by visualization using an EM. Nanogold-labeled Gas7b was prepared for performing cryo-electronmicroscopy according to the protocol provided by the vendor (Nanoprobes). The mixtures of PTX-MTs with or without Gas7b were quickly frozen using ethane slush in liquid nitrogen. Each sample was applied to EM grids, which were examined using a Gatan cold stage in a Philips Tecnai F20 EM operating at 200 kV. The images were photographed on Kodak SO-163 film at a magnification of 50,000× with a defocus of 2,000–2,500 nm.

Size Exclusion Assay—Size exclusion assay was performed by using Superdex 200 10/300 GL (GE Healthcare) in Tris buffer (50 mM Tris-HCl, pH 9.0, 150 mM NaCl). Flow rate was 0.5 ml/min, and 1-ml sample fractions were collected. Gel filtration standard (Bio-Rad) was used as a molecular size standard.

RESULTS

Effect of Gas7b on Cytoskeleton Morphology of Neuro 2A Cells—Fig. 1 shows the morphology of Neuro 2A cells transfected with either control GFP (Neuro 2A-GFP) or GFP-Gas7b (Neuro 2A-Gas7b; Fig. 1). More than 60% cells showed neurite outgrowth in the presence and absence of Gas7b (Fig. 1, B and C). The percentage of Neuro 2A-GFP cells with the neuritis significantly decreased from 60 to 14% after treatment with NOC, an MT depolymerizing agent (Fig. 1C). The percentage of Neuro 2A-Gas7b with the neuritis also decreased from 73 to 31% (Fig. 1C). These results indicated that Gas7b in Neuro 2A cells exerted weak antidepolymerizing effect on neurites in the presence of NOC.

On the other hand, the Neuro 2A-GFP cell morphology was different from that of Neuro 2A-Gas7b cells. Almost all Neuro 2A-GFP cell neurites were slender in shape (Fig. 1). In contrast, Neuro 2A-Gas7b cells were irregularly shaped with dendrite-like branched processes. The dendrite-like processes also comprised by MTs, because they were broken by NOC treatment. These results indicate that Gas7b protein expression in Neuro 2A cells enhance the growth of dendrite-like processes.

Western blot analysis showed that the Neuro 2A cells did not express Tau protein under the growth conditions used in this study (data not shown). Therefore, the effects of Tau on Gas7b were considered to be negligible in this study model (Fig. 1D).

Evaluation of Gas7b on Microtubule Polymerization—In our previous studies, we showed that a low Gas7b concentration (10 nM) increased the turbidity of microtubule-associated protein-containing tubulin solution (13, 20). In this study, we focused...
on the effects of moderate Gas7b concentrations (1–6 μM), because Gas7b overexpression induced alterations in cell morphology. The turbidity-based assay data indicated that Gas7b increases $A_{350}$ in a concentration-dependent manner (Fig. 2). In contrast, the fluorescence-based assay data showed that fluorescence intensities derived from DAPI were not affected by the presence of Gas7b and that treatment with 0.5 μM PTX drastically increased the fluorescence intensity (Fig. 2). These experiments thus produced contradictory results. It should be noted that the turbidity-based assay for MT polymerization involves light scatter measurement and is used to evaluate the numbers and diameters of MTs. On the other hand, the fluorescence-based assay involves the assessment of MT conformation by measuring the fluorescence of a fluorophore (DAPI) whose fluorescent intensity is higher when bound to the tubule conformation of polymerized MTs than to unpolymerized tubulin (24, 25). Co-precipitation assays revealed that the MT cellular levels were not modulated by the presence of Gas7b (Fig. 2C). Pre-incubation of MTs with PTX (PTX-MTs) did not further increase the $A_{350}$ (Fig. 2D). However, Gas7b addition to PTX-MTs solution resulted in increased turbidity in a concentration-dependent manner (Fig. 2D).

Direct Observation of MT Bundles in the Presence of Gas7b—Fig. 3A shows dark field microscopy analysis of MTs in the presence or absence of 6 μM Gas7b. Compared with the control, Gas7b-treated MTs showed thicker filaments and increased granularity. Morphology of PTX-MT in the presence of Gas7b was further analyzed by using an EM (Fig. 3B). Thicker filaments were observed in the Gas7b-treated MTs. The diameter of a canonical filament in the absence of Gas7b was determined to be 25.4 nm (Fig. 3B, left panel), indicating that the filament consists of a single MT (the outer diameter of an MT was ~25 nm). On the other hand, the diameter of filaments in the presence of Gas7b was determined to range between 90.4 and 206.5 nm (Fig. 3B, middle and right panels), indicating that three to five MTs were bundled to form the thicker filaments.

PTX-MTs were incubated with Nanogold-labeled Gas7b and then analyzed by using a cryo-EM (Fig. 3C). A number of Nanogold-labeled Gas7b localized at a spot on MTs surface, which suggested that Gas7b enables binding of MT to other MT as indicated by arrows in Fig. 3C.

Direct Observation of Gas7b-mediated Cross-linking of MTs and F-actin—PTX-MT and F-actin were incubated in F buffer for 2 h at room temperature, and then Gas7b (6 μM) was added. A, after 10-min (left panel) or 30-min (middle and right panels) addition of Gas7b, the mixtures were negative-stained and analyzed by EM. PTX-MTs and F-actin are present as thick and thin filaments, respectively. B, after 30-min addition of Gas7b, Nanogold (0.5 μM) was added to the samples on grids, and the frozen samples were analyzed. Scale bars, 200 nm (A) and 100 nm (B). Closed and open triangles indicate MTs and F-actin, respectively.

Oligomerization of Gas7b, Tubulin, and G-actin—To characterize the molecular interactions of Gas7b with tubulin and
G-actin, Gas7b, Gas7a, and F-BAR domain of Gas7b were prepared (Fig. 5A). A pulldown assay showed that all three Gas7b variants bound to both G-actin and tubulin, indicating that Gas7b bound to these molecules via its F-BAR domain (Fig. 5B).

EM analysis revealed that the F-BAR domain was involved in the MT bundling function (Fig. 3B, right panel). Interactions of Gas7b with G-actin and tubulin were further analyzed by molecular mass estimation of Gas7b-actin, Gas7b-tubulin, and Gas7-actin-tubulin complexes. Size exclusion chromatography analysis was performed using a mixture of Gas7b, G-actin, and tubulin, and fractions were analyzed by SDS-PAGE, which was followed by silver staining for the quantitation of Gas7b, G-actin, and tubulin (Fig. 5, C and D). The molecular size of Gas7b alone was estimated to be in the range of 210–380 kDa (Fig. 5E), indicating that Gas7b forms tetramers or higher order oligomers in solution. Over 80% of tubulin was divided into fractions with the molecular size estimated to be less than 210 kDa (Fig. 5D). The abundance of tubulin was unchanged by the presence of actin. Similarly, the majority of actin solution was fractionated into low molecular size (<65 kDa) fractions, and the abundance of actin was unchanged by the presence of tubulin (Fig. 5, C and D). Large shifts in molecular size of tubulin and actin were observed in the presence of Gas7b, but not in the absence of Gas7b (Fig. 5, C and D). We
observed these tendencies for formation of complex in large molecular size in several experiments.

**DISCUSSION**

Gas7b Enhances MT Bundling but Not Tubulin Polymerization—Gas7b alone did not increase the cellular levels of MTs because Gas7b did not enhance DAPI fluorescence intensities, whereas PTX increased the fluorescence intensities under the same conditions. This finding was corroborated by the co-precipitation assay data, which showed that the amount of precipitated MTs remained unchanged in the presence of Gas7b. Thus, Gas7b did not enhance MT polymerization, but it enhanced the turbidity of prepolymerized PTX-MTs solutions. Therefore, we hypothesized that Gas7b enhances the MT diameter by bundling MTs together to form thick filaments. Dark field and electron microscopy analyses revealed that three to five MTs were bound together in the presence of Gas7b. Use of Nanogold-labeled Gas7b revealed that Gas7b was localized at the junction of MTs. Thus, Gas7b bundles MTs to form thick filaments.

Gas7b Connects MTs and F-actin—Incubation of PTX-MTs with F-actin and Gas7b resulted in F-actin and PTX-MTs linkage. These results revealed that Gas7b connects PTX-MTs to F-actin. The molecular size of tubulin and G-actin increased in the presence of Gas7b. The increase in molecular size could be due to ternary complex formation. Thus, Gas7b may be involved in the regulation of cytoskeleton formation.

We showed that the F-BAR domain of Gas7b was essential for its association with both tubulin and G-actin. F-BAR proteins were shown to form oligomers, and Gas7b was revealed to form a tetramer or a higher order oligomer in solution because PACSIN 1 forms tetramers via its F-BAR domain (26). The oligomeric formation presumably enables a single Gas7b molecule to bind multiple MTs and F-actin by formation of symmetric and multiple binding sites for these cytoskeletons (Fig. 6). Therefore, the oligomeric form is quite important for abilities of Gas7b that bundle MTs and bind between MTs and F-actin.

Gas7b Is Related to Dendrite Development—Dendrite development is a multistage process. The differentiation of granule cells into mature neurons is initiated by neurite outgrowth. During differentiation, the dendrites become polarized and elongated and begin branching dynamically followed by spine growth, which is initiated by filopodia formation. In this study, Neuro 2A cells that did not express Gas7b were arrested in neurite outgrowth stage. Additionally, the cells expressing Gas7b possessed dendrite-like processes and/or filopodia projections, indicating that they advanced to the dendrite branching stage or spine growth stage.

MAP2 (microtubule-associated protein 2) is frequently used as a dendritic marker protein. MAP2-transfected cells were stable against NOC treatment (27, 28). MAP2 is known to stabilize MT growth by cross-linking MTs with intermediate filaments and other MTs; these functions are similar to those of Gas7b. During dendritic filopodia development, F-actin frequently resides on MTs and MT branches in the dendritic shafts (29). Thus, actin-MT cross-linking factors could initiate filopodia formation (30). Thus, Gas7b-mediated bundling of multiple MTs and assembly of MTs and F-actin are both crucial for dendrite development. Thus, we propose that Gas7b expression in Neuro 2A cells regulates dendrite development by facilitating the growth of branch structures and filopodia projections (Fig. 6).

**Biological Implications of Gas7b Expression**—In a previous study, we showed that Gas7b is markedly down-regulated in the brain of AD patients. In this study, we showed that a decrease in Gas7b levels impedes the maturation process of immature neurons by inducing a decrease in MT bundle formation and decreasing assembly of MTs with F-actin.

**Acknowledgments**—We thank A. Fujita, T. Shimizu, K. Kosaka, and K. Kanai for technical support and T. Q. P. Uyeda and N. Umeki for actin preparation.

**References**

1. Squire, L. R., and Zola-Morgan, S. (1991) The medial temporal lobe memory system. *Science* **253**, 1380–1386
2. Bousslama-Oueghlani, L., Wehrle, R., Doulazmi, M., Chen, X. R., Jaudon, F., Lemaigre-Dubreuil, Y., Rivals, I., Sotelo, C., and Dusart, I. (2012) Purkinje cell maturation participates in the control of oligodendrocyte differentiation. Role of sonic hedgehog and vitronectin. *PLoS One* **7**, e49015
3. Zhao, C., Teng, E. M., Summers, R. G., Jr., Ming, G.-L., and Gage, F. H. (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J. Neurosci.* **26**, 3–11
4. Stopa, E. G., Gonzalez, A. M., Chorsky, R., Corona, R. J., Alvarez, J., Bird, et al.
Gas7b Assembles Microtubule and Actin Filament

E. D., and Baird, A. (1990) Basic fibroblast growth factor in Alzheimer’s disease. Biochem. Biophys. Res. Commun. 171, 690–696

5. Hock, C., Heese, K., Hulette, C., Rosenberg, C., and Otten, U. (2000) Region-specific neurotrophin imbalances in Alzheimer disease. Decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampal and cortical areas. Arch. Neurol. 57, 846–851

6. Jin, K., Peel, A. L., Mao, X. O., Xie, L., Cottrell, B. A., Henshall, D. C., and Greenberg, D. A. (2004) Increased hippocampal neurogenesis in Alzheimer’s disease. Proc. Natl. Acad. Sci. U.S.A. 101, 343–347

7. Li, B., Yamamori, H., Tatebayashi, Y., Shafit-Zagardo, B., Tanimukai, H., Chen, S., Iqbal, K., and Grundke-Iqbal, I. (2008) Failure of neuronal maturation in Alzheimer disease dentate gyrus. J. Neuropathol. Exp. Neurol. 67, 78–84

8. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002) Molecular Biology of the Cell, 4th Ed., Garland Science, New York

9. Bhat, K. M., and Setaluri, V. (2007) Microtubule-associated proteins as targets in cancer chemotherapy. Clin. Cancer Res. 13, 2849–2854

10. Ju, Y.-T., Chang, A. C., She, B. R., Tsaur, M. L., Hwang, H. M., Chao, C. C., Cohen, S. N., and Lin-Chao, S. (1998) gas7. A gene expressed preferentially in growth-arrested fibroblasts and terminally differentiated Purkinje neurons affects neurite formation. Proc. Natl. Acad. Sci. U.S.A. 95, 11423–11428

11. Chao, C.-C.-K., Su, L.-J., Sun, N.-K., Ju, Y.-T., Lih, J. C.-J., and Lin-Chao, S. (2003) Involvement of gas7 in nerve growth factor-independent and dependent cell processes in PC12 cells. J. Neurosci. 23, 498–505

12. Lortie, K., Huang, D., Chakravarthy, B., Comas, T., Hou, S. T., Lin-Chao, S., and Morley, P. (2005) The gas7 protein potentiates NGF-mediated differentiation of neurons affects neurite formation. Proc. Natl. Acad. Sci. U.S.A. 102, 32695–32699

13. Akiyama, H., Gotoh, A., Shin, R.-W., Koga, T., Ohashi, T., Sakamoto, W., Harada, A., Arai, H., Sawa, A., Uchida, C., and Uchida, T. (2009) A novel role for hGas7b in microtubular maintenance. Possible implication in Tau-associated pathology in Alzheimer disease. J. Biol. Chem. 284, 32695–32699

14. Lewis, S. A., Ivanov, I. E., Lee, G. H., and Cowan, N. J. (1989) Organization of microtubules in dendrites and axons is determined by a short hydrophobic zipper in microtubule-associated proteins MAP2 and Tau. Nature 342, 498–505

15. Hotulainen, P., and Hoogenraad, C. C. (2010) Actin in dendritic spines. Connecting dynamics to function. J. Cell Biol. 189, 619–629

16. Lippincott, J., and Li, R. (1998) Dual function of Cyk2, a cdc15/PSTPIP family protein, in regulating actomyosin ring dynamics and septin distribution. J. Cell Biol. 143, 1947–1960

17. Greer, P. (2002) Closing in on the biological functions of Fps/Fes and Fer. Nat. Rev. Mol. Cell Biol. 3, 278–289

18. She, B.-R., Liu, G.-G., and Lin-Chao, S. (2002) Association of the growth-arrest-specific protein Gas7 with F-actin induces reorganization of microfilaments and promotes membrane outgrowth. Exp. Cell Res. 273, 34–44

19. Hidaka, M., Koga, T., Gotoh, A., Sanada, M., Hirose, K., and Uchida, T. (2012) Alzheimer’s disease-related protein hGas7b interferes with kinesin motility. J. Biol. Chem. 287, 593–598

20. Uchida, T., Akiyama, H., Sakamoto, W., Koga, T., Yan, K., Uchida, C., Hirose, K., and Itoh, T. J. (2009) Direct optical microscopic observation of the microtubule polymerization intermediate sheet structure in the presence of gas7. J. Mol. Biol. 391, 849–857

21. Castoldi, M., and Popov, A. V (2003) Purification of brain tubulin through two cycles of polymerization-depolymerization in a high-molarity buffer. Protein Expr. Purif. 32, 83–88

22. Noguchi, T. Q., Kanzaki, N., Ueno, H., Hirose, K., and Uyeda, T. Q. (2007) A novel system for expressing toxic actin mutants in Dictyostelium and purification and characterization of a dominant lethal yeast actin mutant. J. Biol. Chem. 282, 27721–27727

23. Gaskin, F., Cantor, C. R., and Shelanski, M. L. (1974) Turbidimetric studies of the in vitro assembly and disassembly of porcine neurotubules. J. Mol. Biol. 89, 737–755

24. Bonne, D., Heusèle, C., Simon, C., and Pantaloni, D. (1985) A fluorescent probe for tubulin and microtubules. J. Biol. Chem. 260, 2819–2825

25. Heusèle, C., Bonne, D., and Carlier, M. F. (1987) Is microtubule assembly a biphasic process? A fluorimetric study using 4’,6-diamidino-2-phenylindole as a probe. Eur. J. Biochem. 165, 613–620

26. Halbach, A., Mörkelin, M., Baumgarten, M., Milbrandt, M., Paulsson, M., and Plomann, M. (2007) PACSIN 1 forms tetramers via its N-terminal F-BAR domain. FEBS J. 274, 773–782

27. Takemura, R., Okabe, S., Umeyama, T., Kanai, Y., Cowan, N. J., and Hirose, K. (1992) Increased microtubule stability and α tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or Tau. J. Cell Sci. 103, 953–964

28. Lewis, S. A., Ivanov, I. E., Lee, G. H., and Cowan, N. J. (1989) Organization of microtubules in dendrites and axons is determined by a short hydrophobic zipper in microtubule-associated proteins MAP2 and Tau. Nature 342, 498–505

29. Korobova, F., and Svitkina, T. (2010) Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. Mol. Biol. Cell 21, 165–176

30. Kajander, P., and Hoogenraad, C. C. (2010) Actin in dendritic spines. Connecting dynamics to function. J. Cell Biol. 189, 619–629