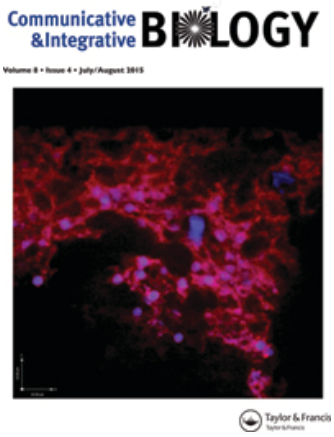

Communicative & Integrative Biology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kcib20

SH2B1 orchestrates signaling events to filopodium formation during neurite outgrowth

Kuan-Wei Chen[a], Yu-Jung Chang[a] & Linyi Chen[abc]

[a] Institute of Molecular Medicine; National Tsing Hua University; Hsinchu, Taiwan, Republic of China

[b] Brain Research Center; National Tsing Hua University; Hsinchu, Taiwan, Republic of China

[c] Department of Medical Science; National Tsing Hua University; Hsinchu, Taiwan, Republic of China

Published online: 31 Aug 2015.

Communicative & Integrative Biology 8:4, e1044189; July/August 2015; Published with license by Taylor & Francis Group, LLC

SH2B1 orchestrates signaling events to filopodium formation during neurite outgrowth

Kuan-Wei Chen[1], Yu-Jung Chang[1], and Linyi Chen[1,2,3,*]

[1]Institute of Molecular Medicine; National Tsing Hua University; Hsinchu, Taiwan, Republic of China; [2]Brain Research Center; National Tsing Hua University; Hsinchu, Taiwan, Republic of China; [3]Department of Medical Science; National Tsing Hua University; Hsinchu, Taiwan, Republic of China

Morphogenesis during development is fundamental to the differentiation of several cell types. As neurite outgrowth marks neuritogenesis, formation of filopodia precede the formation of dendrites and axons. While the structure of filopodia is well-known, the initiation of filopodia during neurite outgrowth is not clear. SH2B1 is known to promote neurite outgrowth of PC12 cells, hippocampal and cortical neurons. As a signaling adaptor protein, SH2B1 interacts with several neurotrophin receptors, and regulates signaling as well as gene expression. Our recent findings suggest that SH2B1 can be recruited to the plasma membrane and F-actin fractions by IRSp53. IRSp53 bends plasma membrane and facilitates actin bundling to set the stage for filopodium formation. We further demonstrate that SH2B1-IRSp53 complexes enhance the formation of filopodia, dendrites and dendritic branches of hippocampal and cortical neurons. While the molecular mechanism underlying filopodium initiation is not clear, we propose that SH2B1-neurotrophin interacting sites may mark the putative sites of filopodium initiation.

Keywords: actin remodeling, filopodia, neurite outgrowth, neuronal differentiation, neurotrophin signaling

*Correspondence to: Linyi Chen; Email: lchen@life.nthu.edu.tw

Submitted: 04/08/2015

Accepted: 04/20/2015

http://dx.doi.org/10.1080/19420889.2015.1044189

Morphogenesis during development involve dynamic and dramatic changes of cell morphology, which is largely regulated by actin cytoskeleton.[1] An array of proteins have been implicated in regulating polymerization of actins into filaments, and thus facilitating the organization of filaments into defined structures, such as lamellipodia, stress fibers and filopodia.[1-5] Filopodia are actin-rich finger-like membrane protrusions and function by sensing environmental cues, serving as sites for integrating signaling and interacting with extracellular matrix. In the developing mammalian brain, neurotrophic factors initiate intracellular signaling to regulate gene expression for controlling neural cell fate. These factors including NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor) and FGFs (fibroblast growth factors) are reported to promote differentiation and survival of neurons.

While neurite outgrowth is the marker for morphological differentiation, filopodium formation precedes the formation of neurites.[6,7] Embryonic hippocampal neurons initiate motile actin-rich filopodia, followed by the microtubule-supported extension of neurites, and then determination of dendrites and axons. Although it is not clear whether the formation of dendrites and axons uses the same mechanism, the initiation and formation of filopodia are obviously important steps during morphogenesis of neurons. Nonetheless, the molecular mechanisms underlying the initial membrane protrusion during filopodium formation is not completely understood.

The adaptor/scaffolding protein SH2B1 is known to enhance neurite outgrowth.[8-12] SH2B1 contains several protein-protein interaction domains that have been implicated in regulating actin, cell motility, differentiation and gene expression (**Fig. 1**). Our recent studies aim to dissect whether SH2B1 participates in the initiation, extension,

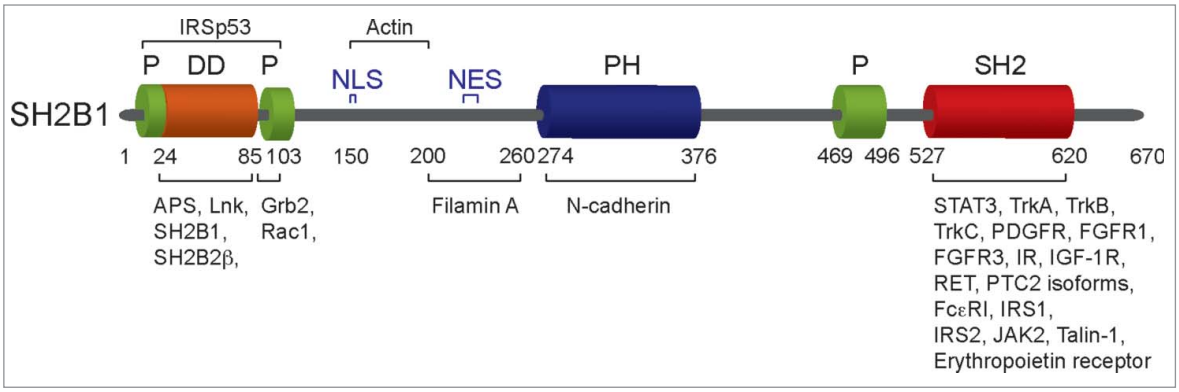

Figure 1. Scheme of SH2B1 domain structure and its interacting proteins. SH2B1 contains 2 N-terminal and a C-terminal Proline-rich domains (P), a dimerization domain (DD), a pleckstrin homology (PH) domain, and a Src homology 2 (SH2) domain. Nuclear localization signal (NLS) and nuclear export sequences (NES) are also identified. The identified interacting proteins to specific domains are indicated. The length of SH2B1 shown is β isoform.

or branching of neurites. An early work suggests that SH2B1 promotes initiation of neurites.[12] We further demonstrate that SH2B1 is recruited to the plasma membrane and F-actin fractions by IRSp53 to promote the formation of filopodia.[13] IRSp53, the Insulin Receptor Substrate of 53 kDa, family has emerged as a dominant regulatory protein for filopodium formation.[14-16] SH3 domain of IRSp53 is known to interact with several actin modifiers (**Fig. 2**). A central question is how IRSp53 regulates cytoskeleton assembly during neuritogenesis. Our findings suggest that SH2B1-IRSp53 complexes promote the formation of dendrites and dendritic branches of hippocampal neurons.[17] Although both SH2B1 and IRSp53 can bind to actin, the actin-binding domain of SH2B1 appears to be not required. Given that SH2B1 and IRSp53 each has several binding partners, it remains to be determined whether their binding partners exist in the SH2B1-IRSp53 complexes and whether they actively participate in the process of neuritogenesis. Moreover, the molecular mechanisms for controlling the initiation of dendritic filopodia are not clear. Dendritic filopodia mainly initiate from the dendritic shaft or small lamellipodia. The actin-rich sites seem to be the base of protruding filopodia. Models for random or signal-induced initiation have been proposed without concrete evidence. Both SH2B1 and IRSp53 are phosphorylated adaptors.[18-20] SH2B1, specifically, is known to undergo neurotrophin-induced phosphorylation at tyrosines, serines and threonines. It is thus very likely that SH2B1 and possibly IRSp53 are phosphorylated in response to external cue of neurotrophins. Subsequently, SH2B1 regulates neurotrophin-induced signaling and gene expression,[8,21] concomitantly being recruited to IRSp53-containing complexes to remodel actin cytoskeleton, and modulate morphogenesis (**Fig. 3**). It is thus reasonable to speculate that SH2B1-neurotrophin receptor interaction sites are at the proximity of SH2B1-IRSp53 complexes which may

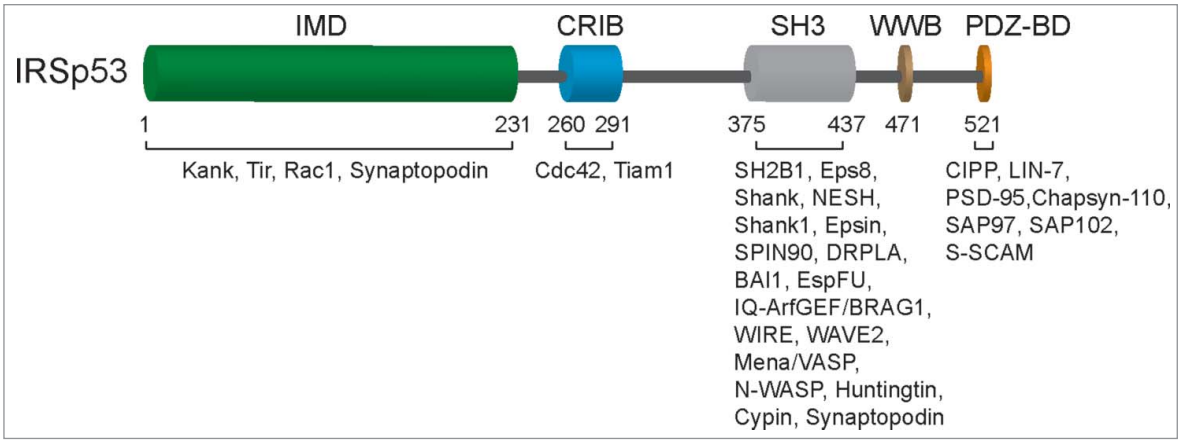

Figure 2. Scheme of IRSp53 domain structure and its interacting proteins. IRSp53 contains several protein-protein interaction domains. The IMD (IRSp53 and missing in metastasis homology domain) domain bends the membrane and promotes protrusion. More than a dozen proteins have been shown to interact with Src homology 3 (SH3) domain of IRSp53. The known interacting proteins to specific domains are indicated.

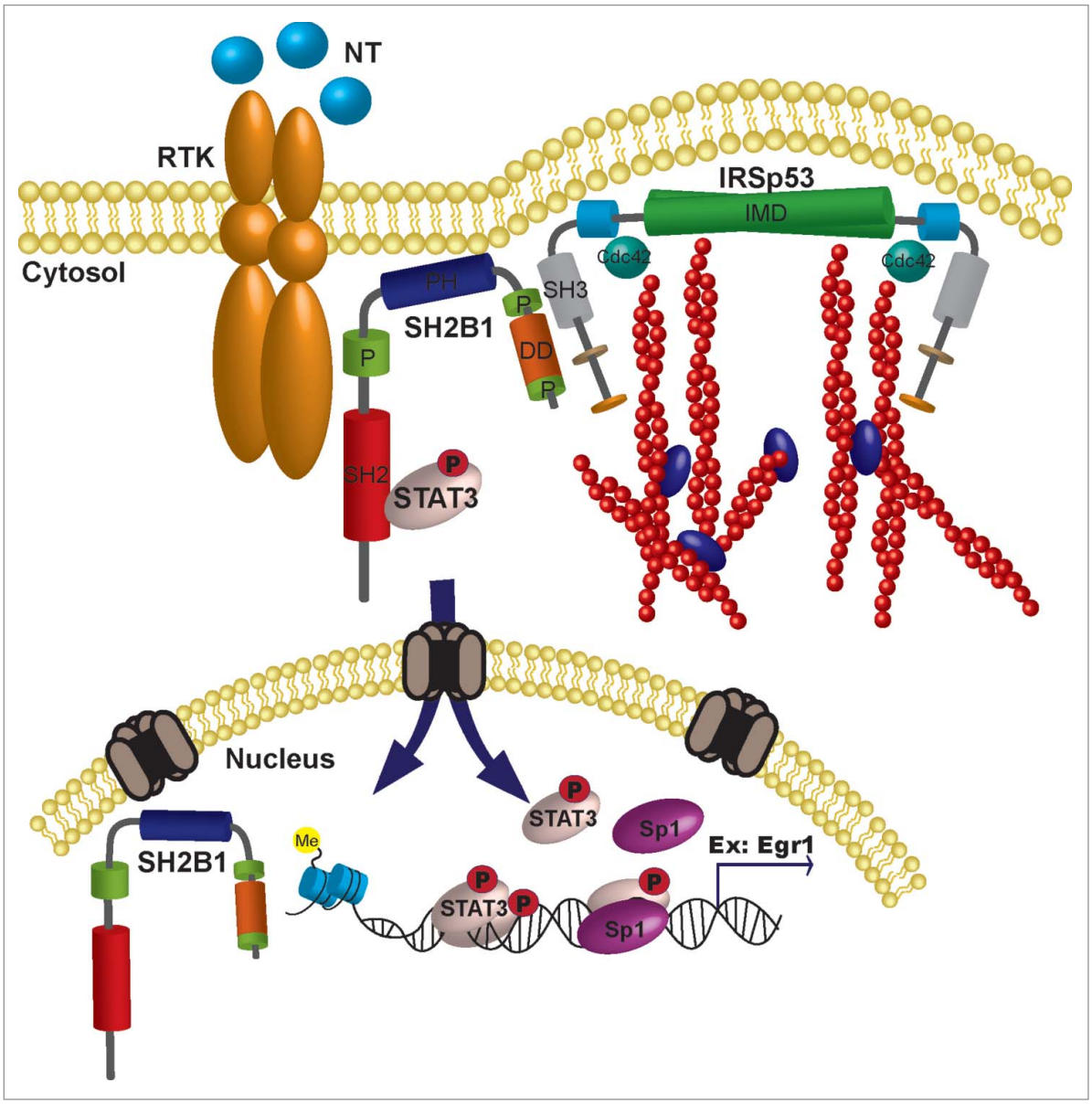

Figure 3. Model for SH2B1 orchestrates signaling and filopodium formation to promote the formation of dendrites in neurons.

mark the putative sites for the initiation of filopodia.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Salbreux G, Charras G, Paluch E. Actin cortex mechanics and cellular morphogenesis. Trends Cell Biol 2012; 22:536-45; PMID:22871642; http://dx.doi.org/10.1016/j.tcb.2012.07.001
2. Paavilainen VO, Bertling E, Falck S, Lappalainen P. Regulation of cytoskeletal dynamics by actin-monomer-binding proteins. Trends Cell Biol 2004; 14:386-94; PMID:15246432; http://dx.doi.org/10.1016/j.tcb.2004.05.002
3. May RC. The Arp2/3 complex: a central regulator of the actin cytoskeleton. Cell Mol Life Sci 2001; 58:1607-26; PMID:11706988; http://dx.doi.org/10.1007/PL00000800
4. Heath JP, Holifield BF. On the mechanisms of cortical actin flow and its role in cytoskeletal organisation of fibroblasts. Symp Soc Exp Biol 1993; 47:35-56; PMID:8165576
5. Nozumi M, Nakagawa H, Miki H, Takenawa T, Miyamoto S. Differential localization of WAVE isoforms in filopodia and lamellipodia of the neuronal growth cone. J Cell Sci 2003; 116:239-46; PMID:12482910; http://dx.doi.org/10.1242/jcs.00233
6. Gallo G. Mechanisms Underlying the Initiation and Dynamics of Neuronal Filopodia: From Neurite Formation to Synaptogenesis. Int Rev Cel Mol Bio 2013; 301:95-156; http://dx.doi.org/10.1016/B978-0-12-407704-1.00003-8
7. Dent EW, Kwiatkowski AV, Mebane LM, Philippar U, Barzik M, Rubinson DA, Gupton S, Van Veen JE, Furman C, Zhang J, et al. Filopodia are required for cortical neurite initiation. Nat Cell Biol 2007; 9:1347-59; PMID:18026093; http://dx.doi.org/10.1038/ncb1654
8. Lin WF, Chen CJ, Chang YJ, Chen SL, Chiu IM, Chen L. SH2B1beta enhances fibroblast growth factor 1 (FGF1)-induced neurite outgrowth through MEK-ERK1/2-STAT3-Egr1 pathway. Cell Signall 2009; 21:1060-72; PMID:19249349; http://dx.doi.org/10.1016/j.cellsig.2009.02.009
9. Chen L, Maures TJ, Jin H, Huo JS, Rabbani SA, Schwartz J, Carter-Su C. SH2B1beta (SH2-Bbeta) enhances expression of a subset of nerve growth factor-regulated genes important for neuronal differentiation including genes encoding urokinase plasminogen activator receptor and matrix metalloproteinase 3/10. Mol

Endocrinol 2008; 22:454-76; PMID:17947375; http://dx.doi.org/10.1210/me.2007-0384
10. Shih CH, Chen CJ, Chen L. New function of the adaptor protein SH2B1 in brain-derived neurotrophic factor-induced neurite outgrowth. PloS one 2013; 8: e79619; PMID:24260264; http://dx.doi.org/10.1371/journal.pone.0079619
11. Rui L, Herrington J, Carter-Su C. SH2-B is required for nerve growth factor-induced neuronal differentiation. J Biol Chem 1999; 274:10590-4; PMID:10187854; http://dx.doi.org/10.1074/jbc.274.15.10590
12. Wang TC, Li YH, Chen KW, Chen CJ, Wu CL, Teng NY, Chen L. SH2B1beta regulates N-cadherin levels, cell-cell adhesion and nerve growth factor-induced neurite initiation. J Cell Physiol 2011; 226:2063-74; PMID:21520058; http://dx.doi.org/10.1002/jcp.22544
13. Hong SJ, Liu ST, Chen CJ, Chen L. SH2B1 increases the numbers of IRSp53-induced filopodia. Biochimica et biophysica acta 2014; 1840: 3335-44; PMID:25175559; http://dx.doi.org/10.1016/j.bbagen.2014.08.011
14. Millard TH, Bompard G, Heung MY, Dafforn TR, Scott DJ, Machesky LM, Futterer K. Structural basis of filopodia formation induced by the IRSp53/MIM homology domain of human IRSp53. EMBO J 2005; 24:240-50; PMID:15635447; http://dx.doi.org/10.1038/sj.emboj.7600535
15. Lim KB, Bu W, Goh WI, Koh E, Ong SH, Pawson T, Sudhaharan T, Ahmed S. The Cdc42 effector IRSp53 generates filopodia by coupling membrane protrusion with actin dynamics. J Biol Chem 2008; 283:20454-72; PMID:18448434; http://dx.doi.org/10.1074/jbc.M710185200
16. Krugmann S, Jordens I, Gevaert K, Driessens M, Vandekerckhove J, Hall A. Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. Curr Biol 2001; 11:1645-55; PMID:11696321; http://dx.doi.org/10.1016/S0960-9822(01)00506-1
17. Chen CJ, Shih CH, Chang YJ, Hong SJ, Li TN, Wang LH, Chen L. SH2B1 and IRSp53 Proteins Promote the Formation of Dendrites and Dendritic Branches. J Biol Chem 2015; 290:6010-21; PMID:25586189; http://dx.doi.org/10.1074/jbc.M114.603795
18. Rui L, Carter-Su C. Platelet-derived growth factor (PDGF) stimulates the association of SH2-Bbeta with PDGF receptor and phosphorylation of SH2-Bbeta. J Biol Chem 1998; 273:21239-45; PMID:9694882; http://dx.doi.org/10.1074/jbc.273.33.21239
19. Rui L, Herrington J, Carter-Su C. SH2-B, a membrane-associated adapter, is phosphorylated on multiple serines/threonines in response to nerve growth factor by kinases within the MEK/ERK cascade. J Biol Chem 1999; 274:26485-92; PMID:10473609; http://dx.doi.org/10.1074/jbc.274.37.26485
20. Okamura-Oho Y, Miyashita T, Yamada M. Distinctive tissue distribution and phosphorylation of IRSp53 isoforms. Biochem Biophys Res Commun 2001; 289:957-60; PMID:11741283; http://dx.doi.org/10.1006/bbrc.2001.6102
21. Chang YJ, Chen KW, Chen CJ, Lin MH, Sun YJ, Lee JL, Chiu IM, Chen L. SH2B1beta interacts with STAT3 and enhances fibroblast growth factor 1-induced gene expression during neuronal differentiation. Mol Cell Biol 2014; 34:1003-19; PMID:24396070; http://dx.doi.org/10.1128/MCB.00940-13