Is Instability Good for the Brain?

Robert A. Carrillo,[1] Kaushiki Menon,[1] and Kai Zinn[1,*]
[1]Broad Center, Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA
*Correspondence: zinnk@caltech.edu
http://dx.doi.org/10.1016/j.neuron.2013.02.002

In this issue of *Neuron*, Nahm et al. (2013) examine the *Drosophila* ortholog of *spartin*, the human gene mutated in a form of hereditary spastic paraplegia. Spartin inhibits BMP signaling and upregulation of BMP signaling may increase microtubule stability and neurodegeneration.

The study of human disease models in genetically tractable invertebrate systems can greatly accelerate progress toward an understanding of the molecular mechanisms underlying these diseases. *Drosophila* has been used to model a variety of human neurological diseases, including Parkinson's disease (PD), fragile X mental retardation, and hereditary spastic paraplegias (HSPs). HSPs are a group of neurodegenerative diseases that are characterized by progressive limb weakness and spasticity. In this issue of *Neuron*, Nahm et al. (2013) have used *Drosophila* genetics to define new linkages between *spartin*, the gene mutated in Troyer syndrome HSP (Patel et al., 2002), and microtubule (MT) stabilization regulated via the BMP signaling pathway.

The *Drosophila* larval neuromuscular junction (NMJ) is a valuable genetic model system for vertebrate synaptic development. Larval NMJ synapses are glutamatergic and use many of the same molecules for neurotransmission and signaling as do excitatory synapses in the vertebrate brain (reviewed by Menon et al., 2013). Nahm et al. (2013) used this system to examine *spartin* function in vivo. Mutations affecting the presynaptically localized *Drosophila* Spartin protein produce an overgrown larval NMJ. Boutons, which are the sites of neurotransmission at *Drosophila* NMJs, are increased in number in *spartin* mutants, and there is also a dramatic increase in "satellite" boutons. Satellite bouton phenotypes are characterized by the presence of small boutons protruding from parent bouton and are thought to arise from defects in synaptic growth. *spartin* mutants also have reduced evoked junctional currents and a lower frequency of spontaneous transmitter release events. These deficits are rescued by presynaptic expression of fly or human Spartin, highlighting the functional conservation between the human and *Drosophila* proteins.

Satellite bouton phenotypes resembling those seen in *spartin* mutants are observed in mutants with presynaptic endocytosis defects, including *Eps15* (Dickman et al., 2006), which encodes a scaffolding protein involved in intracellular trafficking and endocytosis. Eps15 physically interacts with Spartin (Bakowska et al., 2005, 2007) and is responsible for Spartin localization to the presynaptic terminals of the NMJ. Although *Eps15* and *spartin* mutants have similar morphological phenotypes, they do not share the same electrophysiological properties (Koh et al., 2007). These data indicate that Spartin and Eps15 collaborate to control synaptic growth but not synaptic function. The connection of *spartin* to endocytosis was further supported by its genetic interactions with other genes encoding endocytic molecules, including *dap160*, *endophilin*, and *synaptojanin*. In addition, dye uptake experiments directly demonstrated that Spartin contributes to endocytosis.

The *spartin* mutant phenotype and the interaction of Spartin with Eps15 suggest that Spartin affects NMJ bouton development by regulating endocytosis of signaling molecules from the presynaptic membrane. A logical Spartin target is the receptor complex of the BMP signaling cascade, because human Spartin inhibits BMP signaling in cultured cells (Tsang et al., 2009). The BMP receptor ligand Glass bottom boat (Gbb) is secreted by postsynaptic muscles and binds to a presynaptic type II receptor, Wishful thinking (Wit), which forms a tetramer with the type I receptors Thickveins (Tkv) and Saxophone (Sax). The tetramer then phosphorylates mothers against decapentaplegic (Mad). Phosphorylated Mad (P-Mad) binds to Medea, and the P-Mad/Medea complex regulates the expression of genes involved in presynaptic terminal development. The proteins Nervous wreck (Nwk) and Spinster regulate presynaptic BMP signaling by internalization and endosomal trafficking of BMP receptors (O'Connor-Giles et al., 2008; Sweeney and Davis, 2002). Spichthyin, an ortholog of another human protein affected in HSP, NIPA1, controls internalization of Wit (Wang et al., 2007). In both larvae and cultured cells, Spartin was found to also promote Wit internalization and decrease BMP signaling (Figure 1).

Synaptic growth is controlled by MTs, which are stabilized by MT-associated proteins (MAPs). MTs are dynamic structures that drive cytoskeletal rearrangements via cycles of stabilization and destabilization. In a *spartin* mutant, there is an increase in acetylated (stable) tubulin, which is consistent with the observed synaptic overgrowth phenotype. Loss of stable MTs is seen when BMP signaling is decreased (Wang et al., 2007). Since Spartin inhibits BMP signaling, a critical function downstream of Spartin activity could be MT stability. Futsch, a MAP-1B ortholog, was found to accumulate in *spartin* mutants, and *spartin* genetically interacts with *futsch*. Interestingly, when vinblastine, a microtubule-severing drug, was fed to *spartin* mutants, it caused reversion to a wild-type NMJ phenotype.

Futsch expression is repressed by the ortholog of the fragile X mental retardation protein, dFMRP (Zhang et al., 2001). The elevation of Futsch levels in *spartin* mutants suggested that FMRP might be a target of the BMP pathway. Indeed,

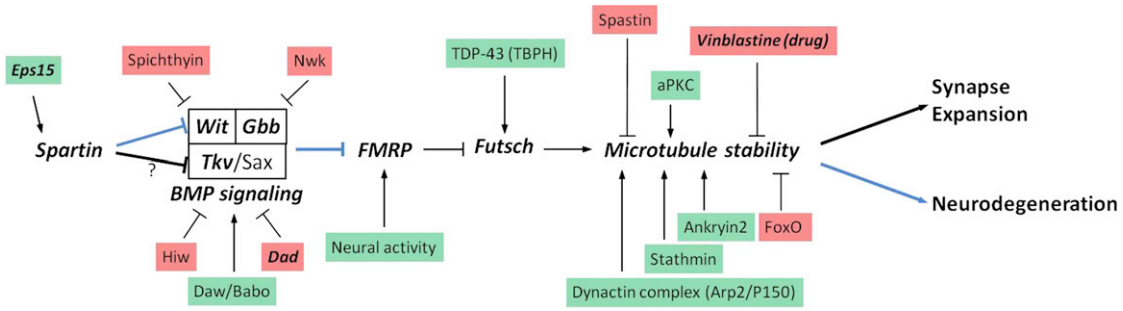

Figure 1. Molecules and Processes Involved in Spartin/BMP/Microtubule Stability Pathways
Red boxes indicate negative influences, and green boxes positive influences. Components examined by Nahm et al. (2013) are in italics, and blue lines represent the novel links they identified. It has not been determined whether Spartin is also involved in endocytosis of other BMP receptors (bar labeled "?"). Other links are from the work of many other groups, not all of whom could be individually cited due to space limitations. Abbreviations: Hiw, Highwire; Daw/Babo, Dawdle/Baboon; aPKC, atypical protein kinase C; FoxO, Forkhead Box protein, class O.

dFMRP protein and mRNA levels were decreased in *spartin* mutants. Mutants lacking an inhibitor of BMP signaling, daughters against decapentaplegic (Dad), have the same phenotypes as *spartin* nulls and have similar decreases in dFMRP expression.

The larval NMJ defects observed in *spartin* mutants suggest that Spartin might be important for development or function of other neural structures. Loss of human Spartin causes the Troyer syndrome phenotype, which includes spasticity of the leg muscles, distal amyotrophy, speech difficulties (dysarthria), and learning impairments. To determine whether *spartin* mutant flies can be used to model the phenotypes of Troyer syndrome, Nahm et al. (2013) performed experiments to test adult flies for locomotor function and neurodegeneration. *spartin* mutants exhibited defects in climbing ability that were rescued by neuronal expression of Spartin. Neurodegeneration was assayed by vacuolization in the brain, and it was found that aged adult *spartin* mutant flies displayed increased numbers of vacuoles. *spartin* brains also have increased numbers of cells expressing markers of apoptosis.

Genetic interactions between *wit*, Dad overexpression, and *spartin* provide evidence that increased BMP signaling is responsible for neurodegeneration in *spartin* mutants. Consistent with this, overexpression of Tkv or Gbb also causes neurodegeneration. P-Mad staining labels apoptotic cells, suggesting that elevated BMP signaling has a toxic effect on neurons.

As at the larval NMJ, the levels of Futsch and acetylated tubulin are elevated in *spartin* mutant brains. Futsch overexpression also produces neurodegeneration. Feeding with vinblastine suppressed the *spartin* neurodegenerative phenotype and partially suppressed the Futsch, Tkv, and Gbb overexpression phenotypes. These findings suggest that increased MT stability in response to upregulated BMP signaling causes neurodegeneration. Interestingly, however, heterozygosity for *futsch* and overexpression also cause neurodegeneration, and vinblastine worsens neurodegeneration in *futsch* heterozygotes, suggesting that reduced MT stability is also toxic. It would be interesting to examine whether elevation or reduction of the levels of Spastin, an MT-severing protein encoded by another HSP gene, would also affect neurodegeneration in wild-type or *BMP/spartin* mutant or overexpression backgrounds.

The work of Nahm et al. (2013) does not specifically implicate dFMRP in adult neurodegeneration. However, FMRP expression is transiently increased after induction of apoptosis in rat brain neurons. Reducing FMRP levels enhances cell death, and FMRP overexpression protects cells (Jeon et al., 2012). If dFMRP is a downstream effector linking BMP signaling to MT stability, translation of neuronal mRNAs that are FMRP targets might be critical in regulating neurodegeneration. Interestingly, mutations affecting TDP-43 (TPBH), an amyotrophic lateral sclerosis-linked RNA-binding protein with roles in mRNA transport and translation, produce a *spartin*-like satellite bouton phenotype, and TDP-43 binds to *futsch* mRNA (Godena et al., 2011). These findings suggest that BMP signaling-induced effects on neuronal protein translation, possibly localized to synapses, could be involved in triggering neurodegeneration.

The Troyer syndrome model described in this issue of *Neuron* by Nahm et al. (2013) highlights once again the power of the *Drosophila* system to contribute to our understanding of the biology underlying human diseases. In future studies, it will be interesting to determine whether the other mutants that have NMJ satellite bouton phenotypes also exhibit neurodegeneration, and whether BMP signaling and MT stability are involved in other neurodegenerative conditions.

REFERENCES

Bakowska, J.C., Jenkins, R., Pendleton, J., and Blackstone, C. (2005). Biochem. Biophys. Res. Commun. *334*, 1042–1048.

Bakowska, J.C., Jupille, H., Fatheddin, P., Puertollano, R., and Blackstone, C. (2007). Mol. Biol. Cell *18*, 1683–1692.

Dickman, D.K., Lu, Z., Meinertzhagen, I.A., and Schwarz, T.L. (2006). Curr. Biol. *16*, 591–598.

Godena, V.K., Romano, G., Romano, M., Appocher, C., Klima, R., Buratti, E., Baralle, F.E., and Feiguin, F. (2011). PLoS ONE *6*, e17808.

Jeon, S.J., Han, S.H., Yang, S.I., Choi, J.W., Kwon, K.J., Park, S.H., Kim, H.Y., Cheong, J.H., Ryu, J.H., Ko, K.H., et al. (2012). J. Neurochem. *123*, 226–238.

Koh, T.W., Korolchuk, V.I., Wairkar, Y.P., Jiao, W., Evergren, E., Pan, H., Zhou, Y., Venken, K.J., Shupliakov, O., Robinson, I.M., et al. (2007). J. Cell Biol. *178*, 309–322.

Menon, K., Carrillo, R.A., and Zinn, K. (2013). WIREs Dev. Biol. http://dx.doi.org/10.1002/wdev.108.

Nahm, M., Lee, M.-J., Parkinson, W., Lee, M., Kim, H., Kim, Y.-J., Kim, S., Cho, Y.S., Min, B.-M., Bae, Y.C., et al. (2013). Neuron *77*, this issue, 680–695.

O'Connor-Giles, K.M., Ho, L.L., and Ganetzky, B. (2008). Neuron *58*, 507–518.

Patel, H., Cross, H., Proukakis, C., Hershberger, R., Bork, P., Ciccarelli, F.D., Patton, M.A., McKusick, V.A., and Crosby, A.H. (2002). Nat. Genet. *31*, 347–348.

Sweeney, S.T., and Davis, G.W. (2002). Neuron *36*, 403–416.

Tsang, H.T., Edwards, T.L., Wang, X., Connell, J.W., Davies, R.J., Durrington, H.J., O'Kane, C.J., Luzio, J.P., and Reid, E. (2009). Hum. Mol. Genet. *18*, 3805–3821.

Wang, X., Shaw, W.R., Tsang, H.T., Reid, E., and O'Kane, C.J. (2007). Nat. Neurosci. *10*, 177–185.

Zhang, Y.Q., Bailey, A.M., Matthies, H.J., Renden, R.B., Smith, M.A., Speese, S.D., Rubin, G.M., and Broadie, K. (2001). Cell *107*, 591–603.

Broadening Roles for FMRP: Big News for Big Potassium (BK) Channels

Anis Contractor[1,*]
[1]Department of Physiology and Department of Neurobiology, Northwestern University, 303 E Chicago Avenue, Chicago, IL 60613, USA
*Correspondence: a-contractor@northwestern.edu
http://dx.doi.org/10.1016/j.neuron.2013.02.001

FMRP is an RNA-binding protein that negatively regulates translation and which is lost in fragile X syndrome. In this issue of *Neuron*, Deng et al. (2013) demonstrate a novel translation-independent function for FMRP as a regulator of presynaptic BK channels that modulate the dynamics of neurotransmitter release.

Fragile X syndrome is a monogenic neurodevelopmental disorder resulting from a trinucleotide repeat expansion in the 5′ untranslated region of the *FMR1* gene. Subsequent hypermethylation of this region leads to silencing of the gene and loss of its protein product FMRP (fragile X mental retardation protein). FMRP has been demonstrated to be an mRNA binding protein that negatively regulates a host of mRNA substrates, likely through mechanisms involving ribosome stalling and through association with microRNAs (Wang et al., 2012). While the complete details of its role in regulating translation are still under investigation, elegant work has identified many of the target mRNAs that are regulated by FMRP (Darnell et al., 2011). FMRP can bind to a large number of mRNAs, but a significant proportion of these encode for synaptic proteins. Based upon this and the many endophenotypes that have been established in *Fmr1* KO mice, the predominant view has been that FMRP is localized to dendrites, close to spines and synapses, where it can rapidly regulate translation of synaptic proteins in an activity-dependent manner. Additionally, there has been a large focus on FMRP-group 1 mGluR interactions at the synapse (Waung and Huber, 2009), further emphasizing the need to understand the postsynaptic roles of FMRP in regulating translation as a way of developing targeted therapeutics.

The study in this issue of *Neuron* by Deng et al. uncovers a novel and unconventional role for FMRP in directly regulating the function of presynaptic ion channels in axons that can ultimately regulate transmitter release (Deng et al., 2013). This is not the first study to propose a role for FMRP beyond the postsynaptic density or spine. FMRP has been localized to growth cones of developing axons (Antar et al., 2006) and has been proposed to have presynaptic roles in establishing synaptic connections (Christie et al., 2009; Hanson and Madison, 2007). Ultrastructural analysis of hippocampal CA1 synapses in *Fmr1* KO mice has revealed a reduction in the length of active zones and a reduced density of docked vesicles in the terminals, all suggesting that FMRP plays a role in the formation of mature presynaptic terminals. Moreover, prior work from the authors of this present study has provided evidence of a functional alteration in presynaptic neurotransmitter release in mature *Fmr1* KO synapses (Deng et al., 2011). In that previous study, trains of stimuli delivered to activate Schaffer collateral synapses in the CA1 of the hippocampus produced greatly augmented responses in *Fmr1* KO mice at stimulation frequencies above 20 Hz and most significantly during stimulation using a natural spike pattern (Deng et al., 2011). This increase in transmitter release was attributed to elevated Ca^{2+} influx during train stimulation in synapses of the knockout mice, although it was not apparent how Ca^{2+} influx through voltage gated channels on the synaptic terminals might be enhanced when FMRP is ablated (Deng et al., 2011).

In the current edition of *Neuron*, Deng et al. follow up on these previous observations by performing a comprehensive and sophisticated group of experiments to identify the mechanisms by which FMRP might regulate neurotransmitter release (Deng et al., 2013). They again use the hippocampal Schaffer collateral synapse, formed between the axons of CA3