Journal Club

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Highwire Balances Synaptic Growth

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Review of Wu et al. (http://www.jneurosci.org/cgi/content/full/25/42/9557)

A growing body of evidence suggests that ubiquitination regulates synaptic structure and function (for review, see DiAntonio and Hicke, 2004). The *Drosophila* protein Highwire (Hiw) contains an ubiquitin ligase domain and acts as a negative regulator of synaptic growth (Wan et al., 2000). In a recent paper in *The Journal of Neuroscience* (Wu et al., 2005), the authors demonstrate that the E3 ubiquitin ligase domain of Hiw is essential for normal presynaptic growth.

The authors examined three Hiw alleles and found that all three exhibit overgrowth of presynaptic terminals at the neuromuscular junction [Wu et al. (2005), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/25/42/9557/FIG2)]. The mutant phenotype was rescued by expressing a wild-type Hiw transgene in the presynaptic motor neuron but not when expressed in the muscle [Wu et al. (2005), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/25/42/9557/FIG3)]. How might Hiw influence presynaptic growth? McCabe et al. (2004) demonstrated that Hiw binds to Medea, a component of the bone morphogenetic protein (BMP) signaling pathway. The BMP signaling pathway plays a major role in regulating the structure and function of the *Drosophila* neuromuscular junction (NMJ). Mutations in components of the BMP signaling pathway lead to small NMJs and deficits in neurotransmission. Hiw and genes of the BMP pathway genetically interact as removal of Medea suppresses the presynaptic overgrowth characteristic of *hiw* mutants. These findings suggest a possible model of presynaptic growth at the *Drosophila* NMJ whereby Hiw regulates synaptic size by controlling the level of presynaptic Medea, the rate-limiting protein in the BMP signaling pathway. When activated, BMP receptors phosphorylate cytoplasmic receptors (Mads), which then form a complex with Medea. The complex then translocates to the nucleus and regulates transcription. In the absence of Hiw, Medea is not degraded and remains available to form complexes with Mad, resulting in continued transcriptional activation and overgrowth of the synapse (Fig. 1).

Although Wan et al. (2000) reported that Hiw is localized to periactive zones, the authors observed only nonspecific synaptic staining using the antibody generated by Wan et al. (2000). In contrast, Wu et al., using a green fluorescent protein (GFP)-tagged Hiw transgene, observed diffuse localization throughout the presynaptic terminal [Wu et al. (2005), their Fig. 3*g–o* (http://www.jneurosci.org/cgi/content/full/25/42/9557/FIG3)]. The authors overexpressed GFP-tagged Hiw, making it possible that the distribution does not reflect the endogenous localization of Hiw. However, localization to the presynaptic cell is in agreement with *in situ* (Wan et al., 2000) and genetic interaction data (McCabe et al., 2004), placing Hiw at the appropriate location to regulate BMP signaling and NMJ growth.

Hiw contains a putative E3 ubiquitin ligase RING domain, which was previously shown to interact with Medea (McCabe et al., 2004). E3 ubiquitin ligases do not possess catalytic activity. Instead, they act as a scaffold by binding to the E2 ubiquitin-conjugating enzyme and the substrate (DiAntonio and Hicke, 2004). Is the RING domain required for normal synaptic growth? To address this question, the authors generated transgenic flies with two cysteine-to-serine mutations in the RING domain (HiwΔRING). These cysteine residues are critical for binding and ubiquitinating the substrate. HiwΔRING mutations failed to rescue the presynaptic overgrowth of *hiw* mutants [Wu et al. (2005), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/25/42/9557/FIG4)], suggesting that Hiw regulates presynaptic growth through ubiquitin-mediated protein degradation. Future work will be required to determine whether Hiw functions as a monomer or as a component of a multimeric complex.

In addition to presynaptic overgrowth, Hiw mutants also exhibit impaired synaptic transmission (Wan et al., 2000). To assess whether Hiw has the same spatial requirements for both the functional and morphological phenotypes, Wu et al. expressed either wild-type Hiw or HiwΔRING in the presynaptic motor neuron of *hiw* mutants. Using intracellular recordings in postsynaptic muscle 6, the authors found that the reduction in spontaneous release, evoked release, and quantal content in *hiw* mutants was rescued by presynaptic expression of wild-type Hiw. Presynaptic expression of HiwΔRING, however, did not rescue [Wu et al. (2005), their Fig. 5 (http://www.

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jneurosci.org/cgi/content/full/25/42/9557/FIG5)], confirming that presynaptic binding of Hiw to its substrate is required for normal morphology and physiology of the synapse. In addition, expression of HiwΔRING in a wild-type background led to a phenotype that resembles the loss-of-function *hiw* mutants [Wu et al. (2005), their Fig. 6*b–e* (http://www.jneurosci.org/cgi/content/full/25/42/9557/FIG6)]. This suggests that HiwΔRING acts as a dominant negative by competing with the endogenous Hiw to affect synaptic size and supports the idea that the RING domain is required for Hiw function.

The *hiw* mutant phenotype is almost identical to the *fat facets* (*faf*) gain-of-function phenotype (DiAntonio et al., 2001). *Faf* encodes a deubiquitinating enzyme that removes ubiquitin from substrate proteins. Together, these studies suggest that the presynaptic motor neuron is regulated by a balance between ubiquitination and deubiquitination of the BMP signaling pathway. In addition, null mutations in *faf* suppress the *hiw* functional phenotype, but not the morphological phenotype, suggesting that the two *hiw* phenotypes are independent. In support of this, Wu et al. observed different temporal requirements for rescue of the functional and morphological phenotypes. Rescue of the morphological phenotype was observed when the wild-type Hiw transgene was expressed in *hiw* mutants throughout development, but rescue was only partial when Hiw was expressed only late in development [Wu et al. (2005), their Fig. 7*a* (http://www.jneurosci.org/cgi/content/full/25/42/9557/FIG7)]. In contrast, expression of the Hiw transgene either throughout development or only late in larval development almost completely rescued the functional phenotype [Wu et al. (2005), their Fig. 7*b* (http://www.jneurosci.org/cgi/content/full/25/42/9557/FIG7)]. Therefore, it appears that there is more than one Hiw substrate responsible for the synaptic phenotype of *hiw* mutants. Hiw interacts with Medea to regulate presynaptic growth (Fig. 1) but interacts with another unknown substrate(s) to affect synaptic function.

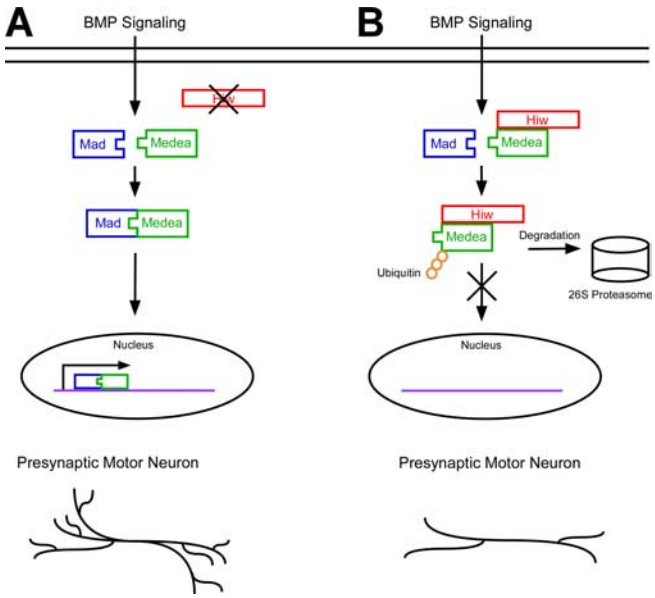

Figure 1. Possible model of *Highwire* regulation of BMP signaling. ***A***, BMP receptors phosphorylate downstream cytoplasmic receptors (Mads), which form a complex with Medea. The complex then translocates to the nucleus to regulate transcription. In the absence of Hiw, more Medea is available to form complexes with Mad, resulting in continued transcriptional activation and overgrowth of the synapse. ***B***, Hiw targets Medea for degradation, reducing the synaptic levels of Medea and negatively regulating synaptic growth.

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