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Ephecting excitatory synapse development

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Abstract

Alterations in synapse number and morphology are associated with devastating psychiatric and neurologic disorders. In this issue of Cell, Margolis et al. (2010) show that the RhoA-guanine exchange factor (GEF) Ephexin5 limits the numbers of excitatory synapses that neurons receive, thus identifying a new mechanism controlling synaptogenesis.

The anatomical and functional basis for communication between neurons is the synapse, a specialized site of cell contact. Synapses consist of a presynaptic terminal, with neurotransmitter filled vesicles, and a postsynaptic terminal containing receptors. Work over the past 10 years has demonstrated a significant role for a number of trans-synaptic adhesion proteins in the process of synapse formation (Dalva et al., 2007). Prominent amongst these are the EphB family of receptor tyrosine kinases. EphBs are required for the formation of normal numbers of excitatory synapses, acting through control of filopodia motility to mediate the formation of these connections during specific developmental times (Dalva et al., 2007; Kayser et al., 2008). Although a number of positive regulators of synapse formation have been described, we know less about the factors that prevent neurons from generating too many contacts. In this issue of Cell an elegant and comprehensive paper by Margolis et al. (2010) shows that the RhoA-guanine exchange factor (GEF) Ephexin5 (also called Vsm-Rho-GEF (Ogita et al., 2003)) limits the synaptogenic activity of EphB2, restricting synapse formation. EphB2, in turn, limits Ephexin5 activity by promoting its degradation by the E3 Ligase Ube3A, relieving the restrictions on synapse formation. Notably, Ube3A is the gene defective in the neurogenetic cognitive disorder known as Angelman Syndrome, which strikes about one in 10,000 live births (Dan, 2009).

Only a small fraction of the contacts between neuronal membranes yield anatomically definable synaptic structures, suggesting that in addition to mechanisms that generate synapses neurons must have ways to restrict synapse formation. Known negative regulators of synapse formation act through a variety of mechanisms. For instance increased neuronal activity, acting through the transcription factor MEF2 (Flavell et al., 2006), and restricted delivery of presynaptic proteins to synaptic sites (Patel and Shen, 2009) can each limit synapse development. Margolis et al. now show that the guanine exchange factor Ephexin5 constrains synapse formation by restricting a specific inducer of synapse formation, EphB2.

Ephexins are a family of five guanine nucleotide exchange factors (GEF) of which only Ephexin1 and Ephexin5 are highly expressed in the brain (Sahin et al., 2005). GEFs control

GTPase activation by catalyzing the exchange of GDP for GTP. When phosphorylated by EphA4, Ephexin1 has potent RhoA activating characteristics, making these GEFs likely mediators of RhoA-dependent reorganization of the actin cytoskeleton in the nervous system. Ephexin1 mediates ephrin-A dependent growth cone collapse and mice lacking Ephexin1 have muscle weakness and impaired synaptic transmission at the neuromuscular junction, likely due to malformation of the active zone (Shamah et al., 2001; Shi et al., 2010). However the function of Ephexin5 has remained obscure.

To identify candidate molecules that might constrain the number of synapses formed downstream of EphB2, perhaps by inhibiting cell motility, Margolis and colleagues first examine the pattern of expression of a number of candidate RhoA GEFs, finding that expression of Ephexin5 matches the pattern of EphB expression. Moreover, in a well controlled series of experiments the authors demonstrate that whereas Ephexin1 interacts selectively with EphA4, Ephexin5 interacts selectively with EphB2 *in vitro* and *in vivo*, has RhoA activating ability that relies on its Dbl-homology domain, and fails to activate either rac1 or CDC-42 GTPases. The RhoA activity in Ephexin5 knockout mice is reduced compared with controls suggesting that Ephexin5 is a major determinant of RhoA levels in the brain.

The authors then use a comprehensive approach to examine the role of Ephexin5 in the control of synapse number. They use shRNA to knock out Ephexin5 in cultured neurons, and also test synapse formation in neurons produced from Ephexin5 knockout mice. In both cases neurons lacking Ephexin5 generate more excitatory synapses compared to controls. In contrast overexpression of Ephexin5 results in a marked decrease in the number of synapses. Importantly these effects depend on the guanine nucleotide exchange activity of Ephexin5. Then, in a clever series of experiments, using brain slices from a conditional Ephexin5 knockout mouse, the authors show that Ephexin5 activity also restricts synapse formation in intact neuronal circuits. Thus, the Ephexin5 GEF limits the number of excitatory synapses neurons make *in vitro* and *in vivo*.

Margolis et al. next show that the effects of Ephexin5 are due to a restriction of EphB2 function during synapse development. Interestingly, although the effects of Ephexin5 on synapse density depend on EphB2 kinase activity, EphB2 activation actually inactivates Ephexin5 by phosphorylation of a specific tyrosine residue, and the inactivation of Ehpexin5 is required for EphB-dependent synapse formation. These results suggest a negative feedback loop, whereby Ephexin5 negatively regulates EphB2, which in turn inhibits Ephexin5 via phosphorylation.

In conducting these experiments, the authors note that the expression level of Ephexin5 is reduced in the presence of EphB2, raising the possibility that Ephexin5 is regulated by proteasomal degradation. In fact, the authors demonstrate that proteosomal destablization of the Ephexin5 protein is tightly regulated by EphB2 *in vitro* and *in vivo*. In cell lines, the expression of EphB2 promotes a decrease in Ephexin5 levels and this effect requires phosphorylation of Ephexin5. Furthermore, a blockade of the proteasome prevents EphB2 dependent degradation of Ephexin5. In vivo, Ephexin5 protein levels are high during times of low synapse formation (P0-P3) and low during periods of rapid synapse addition (P7-P21). However, mRNA levels of Ephexin5 remain constant throughout, consistent with the idea that phosphorylation of Ephexin5 by EphB2 leads to Ephexin5 degradation. Interestingly, previous reports indicate that EphB2 controls synapse formation via regulation of filopodial motility during a similar period of development suggesting that changes in Ephexin5 protein levels are the likely mechanism in initiating or limiting these events (Kayser et al., 2008). Finally, the authors show that Ephexin5 is ubiquitinated in brain

lysates and that it interacts with the E3 ligase Ube3A, which is required for Ephexin5 degradation.

The link to Ube3A is noteworthy because this E3 ligase is defective in 90% of Angelman syndrome cases (reviewed in Dan, 2009). In the current study the authors link Ephexin5 to the etiology of Angelman syndrome using a mouse model of the disease where the maternal inherited copy of Ube3A is deleted ($Ube3A^{m-/p+}$). In brains of these mice, the levels of Ephexin5 expression and the amount of ubiquitinated Ephexin5 protein are increased. Moreover, neurons cultured from these mice are insensitive to ephrin-B1 treatment. In these neurons ephrin-B1 fails to induce reduced levels of Ephexin5 expression. These results lead the authors to suggest that the cognitive defects in Angelman syndrome might results from increased levels of Ephexin5 protein.

Margolis et al. have defined a mechanism that restricts that activity of a specific synaptogenic factor *in vivo* and in functional neuronal circuits. EphB2 initiates synapse development by interacting with specific presynaptic ephrin-B proteins. Ephexin5 suppresses this activity, and EphB2 relieves this repression by phosphorylating and directing Ephexin5 for degradation by the E3 ligase Ube3A. These findings cement EphBs as a key regulator of excitatory synapse development and suggest the interesting possibility that other known synaptogenic factors will have similarly selective restrictive mechanisms. How Ephexin5 acts to restrict EphB2 dependent synapse formation remains unknown, but considering that RhoA activation typically suppresses cell motility these findings suggest that Ephexin5 might limit EphB2 function during synapse formation by down regulating the motility of dendrtic filopodia that EphB2 has previously been shown to mediate. The authors suggest that this may be the case by indicating that Ephexin5 may limit filopodial motility in preliminary unpublished work. Beyond its impact on understanding synapse development, the study provides a tantalizing and exciting potential mechanism to explain the cognitive and behavioral defects in patients with Angelman syndrome.

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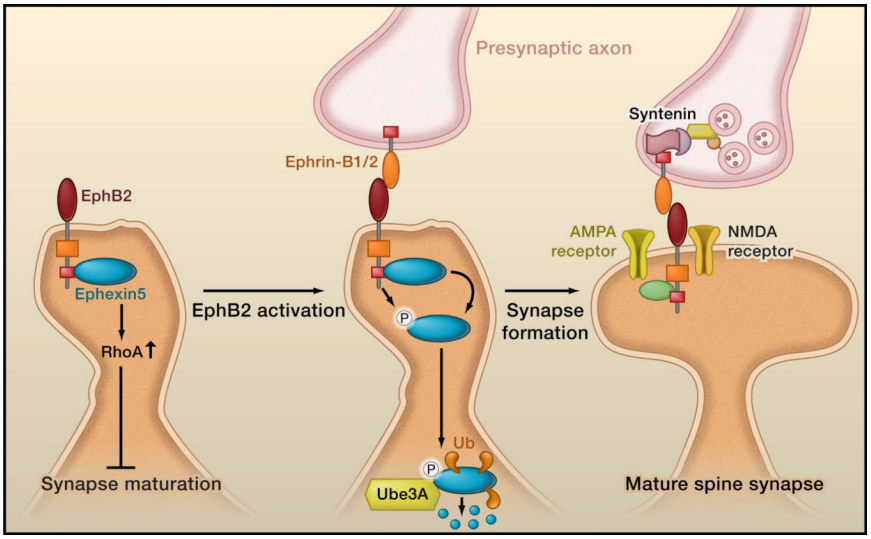

Figure 1. Ephexin5 represses synapse development

Margolis et al. (2010) show that the guanine exchange factor (GEF) Ephexin5 inhibits synapse formation by activating RhoA prior to the activation of the EphB2 receptor by its ephrin-B ligands (left). Once engaged by ligand, EphB2 promotes Ephexin5 phosphorylation, leading to its, ubiquination and degradation by the E3 ubiquitin ligase Ube3A (center). EphB2 can then coordinate synapse maturation by interacting with presynaptic ephrin-Bs, regulating the maturation of dendritic spines and recruiting glutamate receptors (AMPA receptor and NMDA receptor) to the synapse (right).