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Drebrin-Homer Interaction at An Atomic Scale

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In this issue of *Structure*, Li et al. (2018) describe a crystallographic view of the Drebrin-Homer interaction, mediated by the Homer binding motif of Drebrin and EVH1 domain of Homer. This interaction enables cross-linking of monomeric Drebrin by tetrameric Homer, which is required for efficient F-actin bundling.

Drebrin is a protein identified as developmentally regulated brain protein (Shirao and Obata, 1985; Koganezawa et al., 2017 for review). It has an N-terminal actin-depolymerizing factor homology (ADF-H) domain, a coiled-coil (CC) domain, and a carboxyl-tail that comprises nearly half of the protein but does not have any known protein domain and is possibly unstructured (Figure 1). As development progresses, the embryonic Drebrin E form is replaced with the adult Drebrin A form generated by alternative splicing. Drebrin is expressed both in neuronal and non-neuronal tissues. In mature neuronal tissue, it is predominantly expressed in the dendrites and is especially enriched in spines that harbor the excitatory synapses. Overexpression of Drebrin elongates the spines while a knock-down slows synaptic maturation and protein accumulation. Interestingly, overexpression of Drebrin in non-neuronal cells generates protrusions that contain F-actin similar to the dendritic spine. In the knock-out animals, synaptic plasticity and learning capacity are impaired. Therefore, Drebrin is crucial for normal brain function.

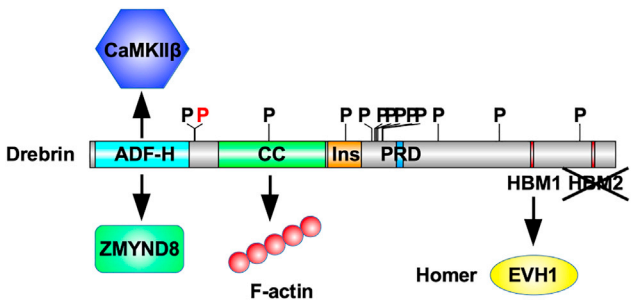

Figure 1. Domain Structure, Phosphorylation, and Protein Interaction of Drebrin
ADF-H, actin depolymerizing factor-homology; CC, coiled-coil; Ins, Drebrin A specific insert; PRD, proline-rich domain; HBM, Homer-binding motif; P, phosphorylation sites; and EVH, ena-vasp homology. While two HBMs have been suggested, a new work by Li et al. (2018) confirmed that only the amino-terminal one is functional. Serine 142 phosphorylation mentioned in the text is highlighted in red. Phosphorylation sites are obtained from mouse Drebrin in UniProt (Q9QXS6).

The proteins that interact with Drebrin are key to its function. Drebrin interacts with F-actin through the CC domain (Figure 1), but not through the ADF-H domain, even though it has homology with ADF, an actin side-binding protein. Instead, the ADF-H domain interacts with ZMYND8, a histone marker reader. Drebrin also interacts with the EVH1 domain of Homer, a postsynaptic scaffolding protein, through PPxxF motifs in the carboxyl tail. To fully understand the functional

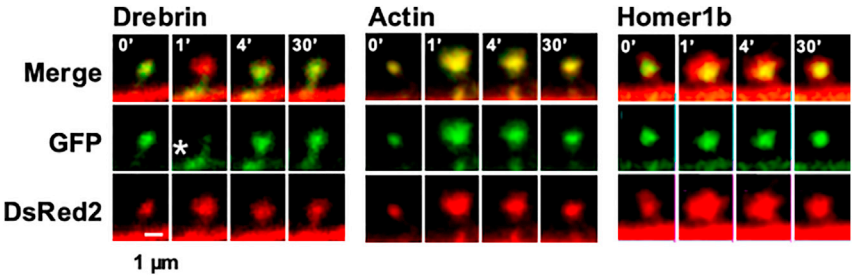

Figure 2. Dissociation of Drebrin from Spine Head during the Induction of LTP
GFP fusion proteins of Drebrin, Homer, and actin were coexpressed with a red fluorescent protein DsRed2 in CA1 pyramidal neurons. Single spine was stimulated by photouncaging of caged-glutamate to induce structural LTP. Asterisk indicates dissociation of Drebrin from spine head and accumulation in the dendritic shaft. Obtained from Bosch et al., (2014) with modifications. Time stamps in min.

significance of these interactions, it is imperative to obtain atomic details.

Mingjie Zhang's group (Li et al., 2018) has addressed this issue by using an X-ray crystallographic approach. Previously, they co-crystalized the ADF-H domain of Drebrin with PHD-BROMO-PWWP domains of ZMYND8 and elucidated the molecular basis of epigenetic regulation of chromatin through histone modification (Yao et al., 2017). Now, in this issue of *Structure*, Li et al. co-crystalized the Homer binding motif of Drebrin with the EVH1 domain of Homer (Li et al., 2018). Although there are two PPxxF motifs on the Drebrin carboxyl tail (Shiraishi-Yamaguchi et al., 2009), only the one toward the N-terminal can interact with Homer. The motif toward the carboxyl terminal was not functional as a Homer binding site. By a systematic analysis of mutants and other known Homer EVH1-binding proteins, Li et al. suggested a more precise motif consensus, L-X_{1-2}-PPXXF-Ω-(D/N). Through this interaction, a tetrameric Homer (long form such as Homer1b) (Hayashi et al., 2009) can cross-link up to four Drebrin molecules. Drebrin alone could not bundle F-actin, though it could bind with it. Only in the presence of tetrameric Homer, efficient F-actin bundling was observed, which is likely due to an avidity effect by cross-linking. In contrast to the tetrameric Homer, a short monomeric form of Homer (such as Homer1a) that possesses only the EVH1 domain prevents the ability of Homer1b/Drebrin to crosslink F-actin. Homer1a is activity-dependently expressed and this effect may serve as a mechanism for Homer1a to downregulate the synaptic strength during homeostatic synaptic plasticity (Hayashi et al., 2012).

How are the Drebrin-mediated interactions regulated? Drebrin is known to be phosphorylated at serine 142, on a linker region between the ADF-H and CC domains (Figure 1), by Cdk5, a kinase implicated in various cellular functions including synaptic plasticity (Worth et al., 2013). Given this, Li et al. (2018) investigated the role of serine 142 phosphorylation by using phospho-block and -mimic mutants. As a result, they found that a phosphomimic mutant has an increased capacity for F-actin bundling. At this point, it is not clear how this phosphorylation works. Worth et al. (2013) suggests that serine 142 phosphorylation unmasks a cryptic F-actin binding region by inactivating the autoinhibitory domain on the carboxyl tail. Furthermore, phosphor-proteomic screening identified additional phosphorylation sites on Drebrin (Figure 1). It will be intriguing to elucidate the mechanism by which serine 142 and other phosphorylation sites change the function of Drebrin at an atomic scale.

How are these protein interactions regulated during physiological processes of synapse? The induction of synaptic plasticity, such as long-term potentiation (LTP) or depression, can modify synaptic protein distribution and dynamics. Indeed, Drebrin, actin, and Homer1b behave very differently (Bosch et al., 2014). Upon activation of an NMDA-type glutamate receptor in a protocol that can induce LTP, actin is rapidly translocated and polymerized into F-actin as the dendritic spine expands (Figure 2). Homer1b does not show changes and is relatively stable. In contrast, Drebrin transiently moves from the dendritic spine into the dendritic shaft, then returns back to the dendritic spine after a few minutes. At this point, we can only speculate what triggers these changes and what the functional significance is. It was recently reported that Drebrin can interact with CaMKIIβ through the ADF-H domain (Yamazaki et al., 2018), which may phosphorylate Drebrin and modulate protein interaction. Indeed, some of the phosphorylation sites, including serine 142, form the consensus phosphorylation site for CaMKII, RxxS/T. Therefore, it is possible that the phosphorylation of Drebrin by CaMKIIβ can modify the protein interaction, which leads to the dissociation of Drebrin from Homer1b. It is unlikely that Homer1a is involved in dissociation because the level of Homer1a in inactive neurons is low and requires some time to be synthesized. Drebrin interacts with F-actin in a manner competing with other F-actin binding proteins such as tropomyosin, fascin, and α-actinin (Koganezawa et al., 2017). Therefore, the dissociation of Drebrin from dendritic spines allows the binding of F-actin with these proteins, which in turn changes the properties of F-actin itself.

The last remaining domain of Drebrin whose structure is yet to be determined is the CC domain. A computer prediction shows that the CC domain can form a dimer while Li et al. (2018) found that Drebrin is a monomer, at least when purified from *E. coli.* This poses an intriguing question of how the CC domain is structured in monomeric Drebrin. It is possible that the CC domain does not actually make a coiled coil as the computer prediction suggests. Alternatively, it may be folded to make an internal CC domain. It would be intriguing to solve this structure as it encompasses the F-actin binding domain. Having said this, I am not excluding the possibility that there are still interesting structural features hidden in the long carboxyl tail or other parts of the protein.

In conclusion, our journey toward a complete understanding of the function and regulation of Drebrin is still on the way. The work by Li et al. (2018) is clearly an important milestone in this journey, which has further illuminated the path for us.

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