Binding of MAGUK family proteins to Neuroligin-1 and -2

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Abstract

Neuroligins (NLs) are cell adhesion molecules (CAMs) that play a vital role in post-synaptic organization. Their intracellular tails contain a principal binding site on their cytoplasmic tails (C-tails), the postsynaptic density protein-95/disks large/zonula occludens-1 (PDZ) ligand, which has been shown to bind with the PDZ domains of membrane-associated guanylate kinase (MAGUK) family proteins. It has recently been shown that, far from straightforward linkage proteins, NLs regulate a wide variety of neuronal processes. A major feature of NLs that allows for versatility is the PDZ binding domain. MAGUKs bind with NL at the PDZ domain and create complex networks of scaffolding proteins within the post synaptic neuron, that determine the organization of receptors as well as other structures in the postsynaptic membrane. However, NL-1 that has its PDZ ligand truncated still displays postsynaptic potentiation of excitatory neurotransmission (Shipman, et al., 2011). NL-1 is specific to excitatory synapses, whereas NL-2 is localized in inhibitory synapses. Notably, the NL-1 and -2 PDZ ligands are identical. We hypothesized that additional binding motifs are present in NL-1 or -2 that give specificity to MAGUK interactions and targeting. We found that NL-1 C-tail lacking its PDZ ligand, NL-1 ΔPDZ, binds to PSD-95 and SAP102. This indicates that another non-PDZ binding motif exists upstream on the C-tail.

Introduction

It has recently been shown that NLs play a critical role in neuronal processes beyond cell-to-cell contact and create complex networks of scaffolding proteins responsible for the organization of glutamate receptors and other post-synaptic density (PSD) proteins. NLs are synaptic CAMs located in the PSD of neurons. A major feature of NLs that allows for functional versatility is the PDZ binding motif. All four NLs contain a four amino acid PDZ ligand that binds readily with the PDZ domains of MAGUK family proteins (Figure 1). NL-1 is almost exclusively expressed in excitatory synapses. Similar to PSD-95, which is solely found at excitatory synapses. In contrast, NL-2 localizes to inhibitory synapses. Yet the NL PDZ ligands are identical (Figure 8). Additionally, NL-1 and NL-3 function normally without the PDZ ligand present (Shipman, et al., 2011).

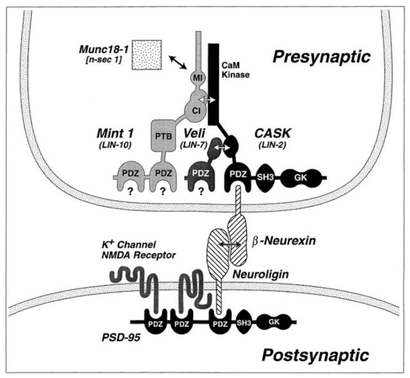

Figure 1. Butz, et al., 1998

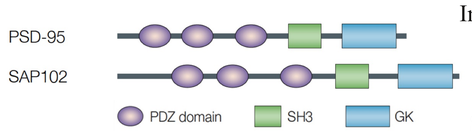

Figure 2. Kim and Sheng, 2004

In recent studies, non-PDZ subunit specific binding has been characterized between 2B and SAP102. Specifically, when the PDZ ligand of the NMDAR NR2B subunit was truncated, PDZ-independent binding was observed with SAP102, but not with PSD-95 (Chen, et al., 2011). To elucidate if additional non-PDZ mechanisms exist in NL-MAGUK binding, we deleted the PDZ ligand of NL-1 and NL-2 and, using a yeast-two hybrid assay, co-transformed the wild type and deletion mutants with MAGUK family members (SAP102 and PSD-95) (Figure 2). We observed whether the resulting binding interaction occurred via the growth of yeast on nutritionally selective plates.

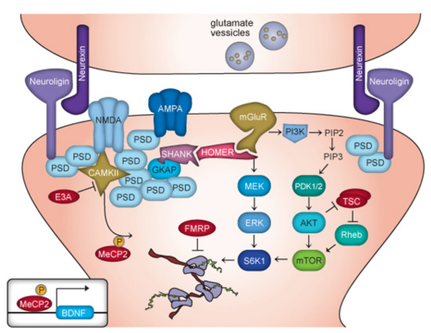

Figure 3. Dölen and Bear, 2009

Results

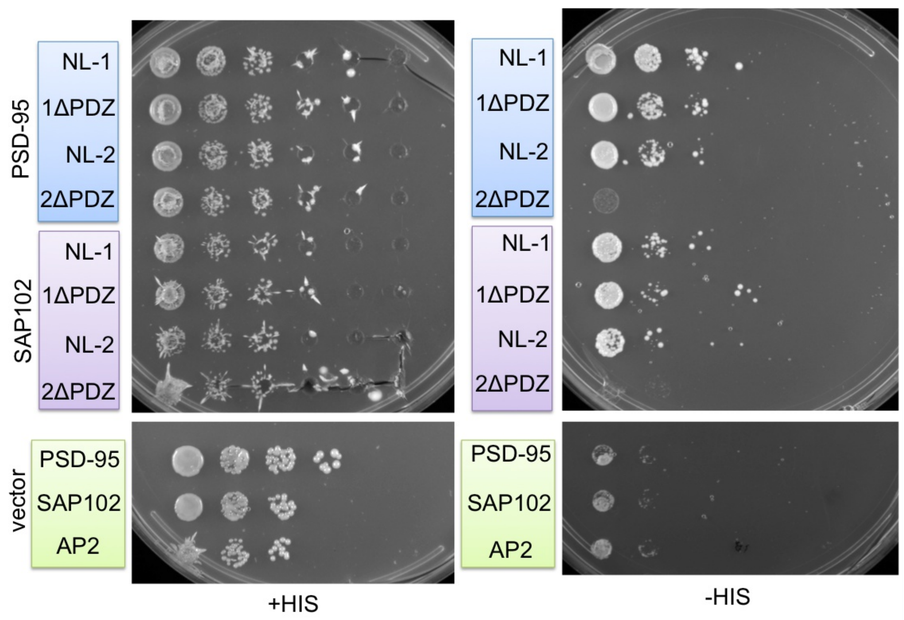

Figure 4. Serial dution of using yeast. On the left is a transformation control conducted on +His plates, the right shows the experimental yeast grown on -His plates. NL-2 ΔPDZ did not grow when co-transformed with the MAGUK proteins (PSD-95 and SAP102). The vector control shows indistinguishable background growth.

The serial dilution revealed that both the wild type C-tails NL-1 and the NL-1 truncation mutant were able to bind with PSD-95 and SAP102. Similarly NL-2 bound with both MAGUK proteins. However, when the PDZ ligand was removed, it was unable to bind. It can be reasoned that NL-1 was able to bind with PSD-95 and SAP102 (without its PDZ ligand) and therefore indirectly reconnect the AD and BD of the transcription protein, whereas NL-2 (when cleaved of its PDZ ligand) had no other alternative binding site to rely on and bound with neither PSD-95 nor SAP102. The transformation control showed equal growth for all samples on the +His plate. The empty vector showed no significant growth. A second trial revealed indistinguishable results.

Figure 9. Cloning Gel, unsaturated

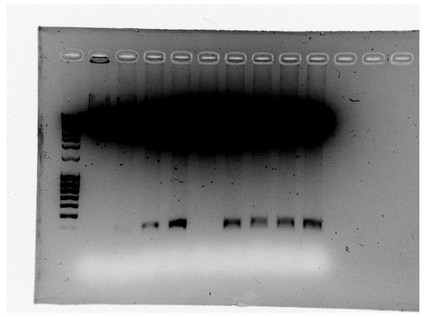

Figure 10. Cloning Gel, saturated

Methods

Cloning

The first step in testing for PDZ-independent binding was to express the NL as a fusion protein both in wild type and ΔPDZ form. This required recombinant plasmid DNA constructs of the NLs as well as minipreps of both. PSD-95 and SAP102 were cloned into the activation domain (AD)-containing vector (pGAD10) and then singly transformed unto nutrient deficient plates to ensure no self-activation occurred. NL-1 and -2 (wild type and ΔPDZ) were cloned into the binding domain (BD)-containing vector (pBHA). *Minipreps and Gel extractions completed using a Qiagen kit.

Yeast two-hybrid

The yeast two-hybrid assay is a technique for studying protein interactions (Figure 7). A transcription factor that would normally transcribe a specific upstream activating sequence (UAS), is cleaved into its respective parts, the BD and the AD. Neither the BD nor the AD will activate transcription of the reporter gene on its own, but only when brought into close contact. In our experiment, NL-1 and -2 C-tails (wild type and ΔPDZ) were fused to the BD whereas PSD-95 and SAP102 were attached to the AD. Transformation of pBHA (the BD containing vector) confers tryptophan-independent growth and transformation of pGAD (the AD containing vector) confers leucine-independent growth. As a negative control, we used an empty pBHA vector. The fifteen combinations were grown on +His or –His (deficient) plates. Growth on –His media indicates a protein-protein interaction where the selective marker was transcribed, showing that the two proteins (NL-1 and PSD-95) are interacting, therefore bringing the BD and AD in close proximity, and driving transcription.

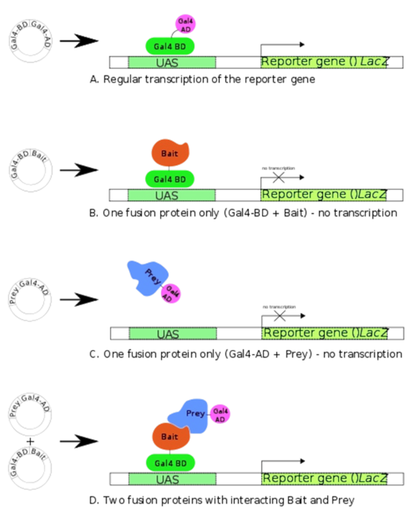

Figure 7. Creative Commons

Discussion

- There is evidence of an alternative non-PDZ binding site on NL-1 for MAGUKs
- The PDZ ligand is not essential for NL-1-MAGUK binding
- This PDZ-independent binding could account for the remarkable specificity of NL-1 vs. NL-2.

The next step for this project will be to locate the alternative binding site on the NL-1 C-tail. These results provide insight into the post-synaptic anchoring mechanisms of NL-1 and -2. Additionally, mutations in genes coding for NLs have been implicated in neurological diseases such as schizophrenia, and autism. Therefore, understanding the precise determinants required for NL targeting to excite or inhibit synapses may lead to further studies for future therapeutic interventions.

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Figure 8. NL Alignment