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SynDIG1 regulation of synaptic AMPA receptor targeting

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Excitatory synapses are composed of several specialized domains including the presynaptic bouton containing several hundred synaptic vesicles (SVs), the presynaptic active zone where SVs dock and fuse with the plasma membrane, and the juxtaposed postsynaptic density (PSD) composed of an electron dense meshwork of proteins including NMDA and AMPA receptors, ion channels, and various signaling components. Cell adhesion molecules (CAMs) extend across the synaptic cleft to stabilize this macromolecular complex. During development of the central nervous system (CNS), certain CAMs also serve as inductive signals that trigger the establishment of pre- and postsynaptic specializations.[1-4] Early events in synapse development include clustering of SVs to the active zone and NMDA receptors to the PSD, whereas later events include targeting of AMPA receptors and synaptic activity that might direct whether synapses will be stabilized, eliminated or strengthened. Regulating the number of AMPA receptors located at the PSD is a key mechanism underlying synaptic strength and plasticity implicated in learning and memory.[5-10] Thus, a current avenue of investigation is the identification of interacting proteins that influence targeting of synaptic AMPA receptors. The discovery that the transmembrane protein stargazin controls synaptic AMPA-R targeting represented a major paradigm shift in the field.[11] My colleagues and I recently reported the discovery of a novel type II transmembrane protein SynDIG1 (Synapse Differentiation Induced Gene I) that functions as a critical regulator of excitatory synapse development in dissociated rat hippocampal neurons.[12] Specifically, knock-down of SynDIG1 in cultured neurons reduces AMPA receptor content at developing synapses by ~50% as determined by immunocytochemistry and electrophysiology.[12] The magnitude of this effect matches that of TARPs and PSD-95 identifying SynDIG1 as a previously unknown central regulator of postsynaptic AMPA receptor targeting. In this addendum I further discuss the implications of these data.

SynDIG1-Mediated AMPA Receptor Clustering

SynDIG1 is present at excitatory synapses and at extra-synaptic sites and cycles between the cell surface and intracellular endosomal compartments.[12] SynDIG1 interacts with AMPA receptor subunits via SynDIG1's C-terminus and this interaction results in co-clustering of AMPA receptors with SynDIG1 in heterologous cells.[12] In these experiments, antibodies against an extracellular epitope for the AMPA receptor subunit GluA2 were applied to heterologous cells under conditions that allow endocytosis of surface-labeled receptors (i.e., after antibody labeling cells were incubated at 37°C for 30 minutes). This implies that SynDIG1-mediated clustering of AMPA receptors occurs upon receptor endocytosis and/or trafficking to an endosomal compartment. Indeed, elimination of the incubation step at 37°C decreased dramatically SynDIG1's ability to cluster AMPA receptors in heterologous cells (Kaur I, Díaz E, unpublished observations), suggesting that internalization is required for SynDIG1-mediated AMPA receptor clustering.

This result in heterologous cells might seem counterintuitive compared with the results in dissociated hippocampal neurons. That is, overexpression or knockdown of SynDIG1 resulted in increased or decreased AMPA receptors at synapses, respectively.[12] Thus, one might predict that SynDIG1-mediated AMPA receptor clustering would occur at the cell surface and/or inhibit AMPA receptor endocytosis to increase synaptic strength. However, recent studies have shown that receptor recycling maintains a pool of mobile surface AMPA receptors that can be delivered to synapses to increase synaptic strength.[13] If SynDIG1 functions to maintain a population of mobile AMPA receptors, then I predict that blocking SynDIG1 function should mimic the effect observed for lateral mobility of surface AMPA receptors when receptor recycling is inhibited (i.e., when endocytic zones are displaced from the postsynaptic region[14]).

SynDIG1 and *Lurcher*

SynDIG1 was identified in a microarray expression profiling screen of mouse cerebellum during postnatal develoopment.[15] In wild type cerebellum, SynDIG1 mRNA is upregulated during postnatal development while SynDIG1 fails to be upregulated in *Lurcher* (*Lc*) cerebellum[15] in which Purkinje neurons degenerate due to a point mutation in the δ2 glutamate receptor (GluRδ2),[16] which is selectively expressed in cerebellar Purkinje neurons.[17] In situ hybridization with antisense probes for SynDIG1 confirmed expression in Purkinje neurons as expected.[12] Purkinje neurons begin to degenerate at postnatal day 12 (P12); however, at P10, prior to Purkinje cell death, parallel fiber-Purkinje neuron synaptogenesis rate is decreased and the ultrastructure of these synapses is defective,[18] suggesting that impaired synaptic maturation due to the *Lc* mutation prior to neuronal death. SynDIG1 expression is reduced in *Lc* cerebellum prior to Purkinje cell death, suggesting that differential expression of SynDIG1 mRNA in *Lc* cerebellum was due to its role in synaptic differentiation of Purkinje neurons.

A further interpretation of SynDIG1 differential gene expression in *Lc* cerebellum is that SynDIG1 might function in the same pathway as GluRδ2 itself. Interestingly, analysis of *GluRδ2*-null mice revealed surprising defects in synapse formation and plasticity between Purkinje neurons and granule neuron parallel fibers.[19-21] More intriguingly, the deficiencies in synapse formation and plasticity associated with *GluRδ2*-null mice phenocopy the defects observed with Cerebellin1 (Cbln1) deficient mice,[22] a member of the C1q/tumor necrosis factor (TNF) superfamily that is expressed and released from cerebellar granule neurons.[23] These data suggest that GluRδ2 and Cbln1 might be involved in a similar pathway to regulate synapse development and plasticity in the cerebellum.[24]

Thus, it is tempting to speculate that SynDIG1 might serve as an auxiliary subunit for GluRδ2 complexes in the developing cerebellum. Alternatively, SynDIG1, as an AMPA receptor interacting protein, might serve as a functional link in Purkinje neurons between AMPA receptor-mediated synaptic transmission and GluRδ2-Cbln1 mediated synapse formation and plasticity. If the former, I predict that *SynDIG1*-deficient mice that have been generated in my laboratory will mimic the synaptic defects associated with *GluRδ2*-null and *Cbln1*-null mice. If the latter, I predict that GluRδ2-Cbln1-dependent synaptic plasticity will require the presence of SynDIG1.

Functional Genomics of Nervous System Development

This study highlights the power of functional genomic approaches to identify genes and to ascribe potential functions based upon their expression profile. The SynDIG1 gene was identified via an unbiased method to analyze gene expression in the developing cerebellum.[15] A functional role for SynDIG1 was predicted based on its differential gene expression in *Lc* cerebellum and the recently published study confirmed a critical role in functional excitatory synapse development.[12] Because SynDIG1 is annotated as a hypothetical protein (tmem90b) in the Genbank database, it is not surprising that it had not been studied previously. Indeed, the expression profiling study identified many genes annotated only as "expressed sequence tags" with similar expression profiles as SynDIG1, suggesting the exciting possibility that the proteins encoded by these genes might also play critical roles in synapse development. Indeed, because genes encoding proteins that function in the same pathway or are in the same complex are often coregulated,[25] it is quite conceivable that the previous expression profiling data will also reveal potential SynDIG1-interacting proteins beyond the previously identified AMPA receptor subunits. Experiments are in progress to test this intriguing possibility.

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