

Summary

The fragrance of flowers, the pain of touching a sharp object, the memory of a holiday, the taste of a rotten apple – all these experiences are made possible by our brain. Human brain is ~1.4kg of tissue composed of billions of neurons that pass information to each other through structures called “synapses”. Synapses are important functional units that determine how information flows through the brain. At synapses neurons communicate with each other by releasing neurotransmitters from one neuron that moves across the synaptic cleft to the next neuron, where they get accepted at specialized sites called receptors. This process is highly regulated and a large number of brain diseases are associated with abnormalities associated with the synapse development and function. Thus, understanding the mechanism, biophysical properties and the molecules associated with synapse development and function is extremely important in finding cure for a large number of diseases.

The research work presented in this thesis covers two aspects of brain development: The mechanism with which Neurobeachin (Nbea), a protein implicated in Autism, targets postsynaptic receptors to the synapse surface. Second half of the thesis deals with Synaptic Cell Adhesion Like Molecule 1 (SALM1), a trans-membrane synaptic protein that is involved in synapse formation in early stages of neuronal development.

In Chapter 2, proteomics was used to identify novel interacting partners of Nbea at the synapse in mouse brain. SAP102, a MAGUK protein implicated in trafficking of AMPA- and NMDA- receptors during synaptogenesis, binds directly with Nbea. However, Immunocytochemistry of endogenous SAP102 and Nbea in cultured neurons does not show co-localization. Nbea interacts with SAP102 via its C-terminal pleckstrin homology domain. Introducing a mutation in PH domain, E2218R, at the C-terminus of Nbea abolishes Nbea-SAP102 binding. Using the E2218R mutation, the mechanism by which Nbea targets postsynaptic receptors to the synapse surface can be elucidated.

In Chapter 3, we used primary neurons from Nbea null mutant mice and rescued these neurons using two Nbea mutants: E2218R Nbea does not interact with glutamate receptor interacting protein SAP102 and Nbea Δ PKA blocks previously characterized interaction with Protein Kinase A Subunit II (PKAII). In Nbea-null neurons, GABA and Glutamate receptors show reduced surface expression, which could be restored by re-expression of full-length Nbea. E2218R Nbea results in loss of binding with SAP102 restored GABA receptor but not glutamate receptor

surface expression in Nbea-null neurons. To understand the relevance of Nbea interaction with SAP102, we analyzed SAP102 null mutant mice. Nbea levels were reduced by ~80% in SAP102 null mice, but glutamate receptor expression was unchanged. An Nbea mutant lacking the PKA binding domain, differentially affected GABA and Glutamate receptor surface expression. Our findings suggest that Nbea targets glutamate and GABA receptors to the synapse via distinct molecular pathways by interacting with specific effector proteins.

In Chapter 4, the role of the Leucine Rich Repeat Cell Adhesion Molecule, SALM1, in synapse development was studied. Large-scale mass spectrometry analysis of SALM1 reveal several synaptic proteins out of which CASK binds directly with SALM1 in heterologous cells. To determine endogenous distribution of SALM1 in cultured neurons, a specific SALM1 antibody was developed which shows that SALM1 is apposed to ER and Golgi and is present in a subset of glutamatergic synapses. To understand the role of SALM1 in synaptogenesis, SALM1 was knocked down at different stages of synapse development using short hairpin RNAs. SALM1 depletion at 7 days in vitro (DIV7) or earlier, resulted in a 60% reduction of excitatory synapses, while infection of SALM1 shRNA after DIV9 had no effect on synapse formation. This phenotype could be rescued with shRNA resistant SALM1. We conclude that SALM1 is required for the development of excitatory synapses but dispensable for synaptic maintenance.

In Chapter 5, we explored the mechanism by which SALM1 supports synapse formation and if the protein plays a role in synaptic transmission. Patch clamp analyses on single isolated neurons, infected with SALM1 shRNAs at DIV7 and at DIV9 shows that residual synapses in SALM1 depleted neurons are physiologically normal. Removal of SALM1 unilaterally at the pre synapse or post synapse does not alter the kinetics of synaptic vesicle release or postsynaptic receptor surface expression. Most importantly, synapse numbers are not reduced with this unilateral reduction of SALM1. Our data suggest that SALM1 is sufficient at one side of the synapse for its role in synapse development.

In Chapter 6, possible pathways with which Nbea interacts with other molecules to target postsynaptic receptors to the synapse are discussed. A model is provided for the mechanism with which Nbea performs its function. Secondly, function of SALM1 in synapse formation is discussed in broader context of cell adhesion molecules role in synaptogenesis. Based on finding in Chapter 4 and Chapter 5, a model is generated with which SALM1 interacts with other proteins in the biosynthetic pathway and at the synapse for synapse formation.