Abstract:

Normal formation of synapses requires the transport, recruitment, and stabilization of the synaptic vesicle-regulating protein synapsin to sites of developing presynaptic terminals. Recruitment of synapsin to nascent synapses is regulated by cyclin-dependent kinase 5 (Cdk5), but the downstream effectors of Cdk5 that enable this recruitment remain unknown. Using a zebrafish model, our research has examined a putative role of the scaffolding protein calcium/calmodulin-dependent serine kinase a (CASKa) in synapsin recruitment. This line of research was motivated by several observations:

- The mammalian ortholog CASK participates in multipartite cargo transport complexes.
- Localization of mouse CASK to presynaptic terminals is dependent on Cdk5-mediated phosphorylation.
- CASK can by phosphorylated by Cdk5 at serine residues 51/395.

These observations spurred our hypothesis that Cdk5 phosphorylates CASKa to recruit synapsin to presynaptic terminals. Using a stereotypical touch-evoked behavior to assess synapse function, we found that embryos misexpressing non-phosphorylatable CASK protein exhibited a significant reduction in touch response when compared to embryos expressing either endogenous CASKa or exogenous wild-type CASK protein. This behavioral data suggest synapses from touch-sensitive neurons are compromised under these conditions. We now wish to characterize the molecular and synaptic basis of the touch deficit using immunofluorescent probes for synapsin and other synaptic markers. As aberrant function of human CASK is linked to defects in synapses and a host of neurodevelopmental disorders, including microcephaly and X-linked intellectual disability, this examination may provide further insight into the machinery of synapse formation, as well as uncover a novel target for CASK-associated disorder remediation.

Methods:

Synapses are units of connectivity in the nervous system, each permitting the transmission of information between neurons. Synapse formation requires that the necessary proteins be synthesized, transported, and deposited at sites of pre- and postsynaptic assembly. To dissect this mechanism in developing zebrafish, we have employed biochemical techniques, behavioral assays, immunofluorescence, and confocal microscopy.

Previous studies motivated our hypothesis that Cdk5 acts on CASKa to recruit synapsin to presynaptic terminals. Synapsin is a molecule that is necessary to regulate the active pool of synaptic vesicles at mature synapses. In testing this hypothesis, we first investigated the phenotypic effect induced by aberrant expression and mutation of CASK.

In the embryonic spinal cord, synapses between Rohon-Beard (RB) sensory neurons and commissural primary ascending (CoPA) interneurons are sufficient to mediate touch-evoked coiling behavior by 25 hours post fertilization. We use this stereotypical touch-evoked behavior to assess synapse function in embryos, under the assumption that robust touch response corresponds to robust synapse function.

To knock down expression of endogenous CASKa, zebrafish embryos at the 1-2 cell stage were injected with a splice-blocking morpholino targeting CASKa RNA transcripts. CASKa-knockdown embryos exhibited a significant reduction in touch response when compared to control embryos. Touch response of CASKa-knockdown embryos were rescued by co-injection of wild type mammalian CASK RNA. Next, a CASK RNA construct was mutagenized to encode the non-phosphorylatable amino acid alanine at residues 51/395, thereby encoding a CASK protein unbridled from Cdk5 regulation. Embryos misexpressing this non-phosphorylatable CASK protein exhibited a significant reduction in touch response when compared to embryos expressing either endogenous CASKa or exogenous wild-type mammalian CASK protein. Notably, embryos misexpressing the mutant CASK without a concomitant knockdown of endogenous CASKa exhibited a significant reduction in touch response. This result

suggests non-phosphorylatable CASK exerts a dominant negative effect on synapse assembly or function.

Although these behavioral results support our hypothesis qualitatively, a quantitative analysis of synapse formation will bolster this study. Next, we wish to examine the synapsin fluorescence immunolabeling at presynaptic terminals of RB-CoPA synapses and compare intensities among the experimental groups. To do this, embryos from each condition must be labeled with immunofluorescent probes for synapsin and the post-synaptic marker panMAGUK. Labeled embryos can then be mounted for visualization by laser confocal microscopy. We anticipate that synapsin localization at presynaptic terminals will be decreased in embryos from both the CASKa-knockdown and CASK mutant conditions when compared to control embryos.

Despite the utility of morpholinos, there is a drawback to their experimental use, as they can cause off-target effects on irrelevant genes. The rescue experiment, in which exogenous CASK mRNA is injected into the CASKa-knockdown embryos, addresses the problem of off-target gene effects, because such embryos have their functional phenotype rescued. To further control for off-target effects, we will immunofluorescently probe for synaptotagmin2b, another presynaptic marker, using the znp-1 antibody. If the CASKa morpholino is affecting synapse formation in no other way than hypothesized, we expect to see a near-equal distribution of synaptotagmin2b at presynaptic terminals in embryos from all conditions.