Identification of amphiphysin 1 as an endogenous substrate for CDKL5, a protein kinase associated with X-linked neurodevelopmental disorder. Archives of Biochemistry and Biophysics 2013.

The molecules on which any enzyme acts are known as its substrates and you will remember that CDKL5 is a kinase that energises other molecules through a process called phosphorylation. Identification of substrates that are phosphorylated by CDKL5 will help in the understanding of its role and function. Previous studies have so far identified 2 substrates, MeCP2 and DNA methyltransferase 1 (Dnmt1). In this paper from Japan, the authors describe a new technique with which they were able to identify a further substrate - amphiphysin 1 (amph 1) - a protein coded by a gene on chromosome 7 which is associated with the cytoplasmic surface of synaptic vesicles and is thought to have important roles in neuronal transmission and development.

The bulk of the paper describes specific details of the various stages of the technique which are fairly technical (for me anyway!). However, the bottom line is that amph 1 appears to be a much stronger substrate than either MeCP2 or Dnmt1, and by this, the authors mean and show that amphi 1 is strongly phosphorylated by CDKL5. Previous studies have shown that CDKL5 is localized not only in the nucleus but also in the cytoplasm, especially in the early developmental stages. Amph1 is also known to be present in the cytoplasm in significant amounts and has an important role in neuronal function. It is therefore suggested that phosphorylation of Amph1 in the cytoplasm by CDKL5 plays a crucial role during the early stage of neuronal development.

In order to further show that amphi 1 may be a substrate of CDKL5, the authors created 3 "mutation" proteins. Each protein had a point (missense) mutation that has been described in the literature in children with a CDKL5 disorder. The mutations were within the kinase domain of the protein and each of these 3 "mutated" proteins failed to show any phosphorylating activity towards amphi 1. The authors therefore conclude that Amph1 is a potential endogenous substrate for CDKL5 and that it is potentially a critical molecular component of the pathogenesis underlying CDKL5-related neurodevelopmental disorders.

Note - more technical stuff!! The term "endogenous" is important here as enzymes (proteins) can show far wider activity in the laboratory (in vitro) than they actually have in the body (in vivo). We are clearly more interested in endogenous substrates and it may therefore be necessary for this study (and other in vitro studies) to be corroborated in vivo by perhaps, using knockout or knockin mouse technology.