Regulation of neuron growth and development by the matricellular protein dCCN

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Regulation of neuron growth and development by the matricellular protein dCCN
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1 – Introduction

The extracellular matrix (ECM) provides critical biochemical and physical signals to initiate and support a diverse array of cellular functions. The CCN (CCN1, CCN2, CCN3) family of proteins functions in a primarily regulatory role rather than structural. CCN members are highly expressed in the central nervous system and contribute to neuron development, differentiation, and synaptic plasticity. However, the contributions of CCN family members and matricellular proteins to neuron communication and synaptic transmission remain understudied. Here we examine the role of the sole Drosophila CCN (dCCN) family member in the developing and mature nervous system. dCCN is expressed in motorneurons and interneurons throughout embryonic, larval, and adult stages. In the adult, dCCN expression is found in peripheral neurons located in the legs, wing, and proboscis that respond to sensory information and in the ventral nerve cord in neurons that innervate the reproductive system and motorneurons. In the central brain, our results demonstrate dCCN is expressed in octopamine (OA) neurons, dopamine (DA) neurons, and neurons that express the male form of Fruitless (FruM), a key regulator of male-specific behavior.

Hypotheses:
- dCCN is required for neuron function
- Loss of dCCN will result in alterations to neuron communication and changes in behavior

The dCCN protein has three of the four well conserved domains.

(A) The four well-conserved domains of CCN. (B) The three well-conserved domains found in dCCN. Insect-like growth factor binding protein (IGFBP), a von Willebrand factor type C repeat (VWC), thrombospondin type 1 repeat (TSP)-1, and a cysteine knot-containing domain (C K) C) The many interacting partners of the CCN and dCCN domains. Bone morphogenetic protein 4 (BMP4), transforming growth factor β (TGFβ), lipoprotein receptor-related protein 1 (LRP1), and Heparin sulfate proteoglycans (HSPG).

2 – Tools to examine CCN in Drosophila melanogaster

To manipulate gene expression, the Gal4/UAS binary system will be utilized. The Gal4/UAS system is used every day in Drosophila labs around the world to activate, knockdown, or inhibit gene expression. Gal4, a yeast transcriptional activator, is expressed under control of a cell or tissue-specific promoter. Upon expression, Gal4 binds to the upstream activating sequence, UAS, to activate transcription of the gene or RNAi sequence downstream of the UAS sequence. To reduce dCCN function, a Drosophila dCCN mutant line was generated in the Certel lab. This line contains an insertion of Gal4 into the coding region of dCCN. The resulting line drives Gal4 expression under the dCCN promoter thus faithfully recapitulating endogenous expression patterns, as well as creating a loss-of-function allele due to disruption of the dCCN coding region.

3 – dCCN expression

Drosophila melanogaster undergoes morphological changes throughout development within their life cycle, which is comprised of 4 major stages: embryo, larval, pupal, and adult stage.

4 – dCCN expression

dCCNGal4 is expressed in FruM, OA, and DA neurons. FruM neurons are required for male behavior such as courting or aggressive behavior. Previous work of the Certel lab has demonstrated that OA null mutants show a selective loss of high and low levels of aggression and high levels of courtship. These findings suggest a role for dCCN among amine neurons, differentiation, and/or function of sex-specific circuitry. We next examined aggression as a readout for neuron function.

5 – Loss of dCCN results in decrease in aggressive behavior

Using aggression as a readout for neuron function, decreased levels of aggression are observed when dCCN is lost or reduced.

(A) dCCNGal4 is expressed in the majority of FruM expressing neurons, including the Kenyon cells that comprise the mushroom bodies (arrow, UAS-dCCNGal4/FruM-Hs-GFP). (B) The majority of OA neurons (arrows) co-express dCCN (dCCNGal4/UAS-dsRed with anti-tac2 staining). (C) dCCNGal4 is expressed in some DA neurons (arrows) distal of the mushroom body area (dCCNGal4/UAS-dsRed with anti-TH staining).

6 – Conclusions

How does the loss of dCCN affect neuron function and behavioral circuitry in Drosophila melanogaster?
- dCCNGal4 expression identified throughout developmental stages in many tissue types
- dCCNGal4 expressed in FruM, OA, and DA neurons
- Loss of dCCN results in changes in male aggressive behavior
- dCCN critical for development and neuron function

Future Directions
- Examine changes in aggression when dCCN is reduced in specific subsets of neurons
- Identify and quantify expression of dCCN in other amine neurons such as seroton
- Examine subsets of dCCN neurons to investigate morphology and synaptic connections

Special thanks to David Hess-Homeier for construction of the dCCNGal4 line, and Jessie Bailey for dCCN transcript level data.

(A) An increase in latency, and a decrease in lunges, wing threats, and male-male wing extensions are observed in dCCN–RNAi lines and homozygous dCCNmutant flies (dCCNGal4/+;dCCN–RNAi, dCCNGal4/+;dCCNmut). (B) An increase in latency and male-male wing extensions, along with reductions of lunges and wing threats is observed when dCCN is reduced in OA neurons (UAS-dsRed-gal4/; UAS-dCCN-RNAi). (A-B) Aggression data sets for latency, lunging, wing threats, and male-male courting wing extensions. A Kruskal-Wallis multiple comparisons non-parametric test was used to determine statistical significance. Error bars report SEM. * equals p<0.05, ** equals p<0.01.