Effects of Injury on Neuronal Synaptobrevin (n-syb) in *Gryllus bimaculatus*
Dennis Zambrano, Class of 2017

Damage to the nervous system of many organisms usually results in damage and degeneration of the affected neurons. However, the cricket *Gryllus bimaculatus*, exhibits a compensatory mechanism that allows neuronal growth after nervous system damage. A gene that is involved in this compensatory mechanism is neuronal synaptobrevin. Neuronal synaptobrevin (n-syb) has previously been shown to exist in two forms; a short truncated form, and a full-length form. The relative abundance of each form is hypothesized to change during the compensatory growth mechanism. One of methods of detecting different forms of a gene being expressed is called *in situ* hybridization (ISH). Traditionally ISH employs a color reaction that produces a dark stain where the targeted gene is expressed. However, since the short form is identical to the long form up to the point at which it becomes truncated, targeting the two different forms of n-syb (which have a great degree of overlap) is very complicated with traditional methods. This problem can be circumvented with a new variant of ISH called Hybridization Chain Reaction (HCR). The advantage of HCR over traditional ISH is its capability of detecting multiple genes expressed in a single tissue. HCR uses fluorescence of different colors rather than a dark stain, thus different genes, or different forms of genes, can be selectively detected and visualized. Another advantage of HCR is its ability to amplify the existing signal. Once the gene of interest is detected, specific molecules called amplifiers initiate a reaction with the detected gene to enhance the signal. Since these amplifiers contain the fluorescence, and they are highly specific to the gene of interest, there is very little background staining.

HCR is a new tool that can be advantageous in our study of the differential regulation of n-syb (short vs. long), however, the cricket we work on is not a model organism. Thus, in order to be able to use HCR in our organism, it was important to first practice with traditional ISH and to develop or adapt our existent ISH protocol for HCR. To practice ISH we used the gene expression of a previously studied guidance molecule called Sema2a. Sema2a has already been characterized in embryonic, brain, and prothoracic ganglia tissue of the cricket. Thus, it seemed an ideal candidate in which to replicate experiments. In our experiments we obtained the expressions described by different authors. In addition, from our experiments we learned of the importance of different chemicals, reagents, and experimental conditions (eg. pH or temperature) that are essential for a successful ISH. With this knowledge, and with the help of the company developing the probes, we determined that the best way to adapt our protocols to HCR would be to use our existing protocols up to the pre-detection stage. At this point we would follow the manufacturer's suggested protocols. This was decided based on how the tissue of our non-ideal system is obtained and processed. For example, HCR has previously been done on zebra fish embryos without an enzyme treatment to allow reagent penetration. However, in our tissue enzyme treatment is key to success of traditional ISH. In consequence it seemed reasonable to keep that step in our adapted protocol.

In the end, by the time our reagents were synthesized and delivered my fellowship had come to an end. It wasn't possible to start an experiment since HCR is a multiple day procedure. Regardless, with an idea of a protocol to follow, and with the experience obtained performing ISH, performing an HCR doesn't seem as daunting as it once did. The reagents will be kept in the laboratory for future use.

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