In the cricket, *Gryllus bimaculatus*, a remarkable and precise mechanism of auditory regeneration exists. When the cricket’s auditory neurons are deafferented, achieved by severing the front leg, they redevelop and form functional connections to the auditory nerves on the other ear. This mechanism restores the cricket’s hearing and balance. In the past, the Horch Lab has examined why and how the cricket regrows its nerve dendrites by looking at changes at the molecular level: perhaps the genes in the cricket are expressing different proteins during the restoration process. Thus, the lab used Subtractive Hybridization to compare and contrast what was changed during this process. 49 gene candidates were identified as down-regulated, with no significant result for up-regulation. Unfortunately, a database search on these candidates revealed that 55% of them did not have significant matches that may indicate what their functions may be in the cricket (Horch et al).

The purpose of my summer research project is to obtain a more sequence for some of these gene candidates and do another database search on them to see if I can find any significant matches. This is accomplished by using a Polymerase Chain Reaction (PCR) method called Rapid Amplification of cDNA Ends (RACE). In summary, PCR can make millions of copies, or amplify, a desired gene sequence in few hours. RACE is a method used when only part of the full sequence is known. RACE not only allows me to amplify the candidate genes, but also allows me to obtain a more complete sequence. The procedure begins with synthesizing RACE cDNA, which will serve as the DNA template for the reaction where the copies will be made on. The cDNA is made through transcribing RNA from the cricket, and the pieces are then tailored through the use of enzymes and primers, which results in cDNA templates for RACE. Then, through the use of primers designed based on the gene candidates, using an online program called Primer3, the RACE reaction builds the copies from the known sequence as located by the primers to the ends of the full sequence. The products are then run on an electrophoresis gel to check whether or not the RACE reaction was a success (Matz et al).

RACE is a perfect technique for my project because the products from the protocol should be full length sequences of the candidate genes. Having more sequence from these short known candidates would allow for more results on gene function from the database search, since having more sequence increases the specificity of what the sequence could code for and the database would have more data to match the sequence with.

Throughout the summer, I have mainly worked with four gene candidates: C3B6, Pushover, Ubiquitin Protease, and Transcription Initiation Factor. Of the four candidates, C3B6 has the highest priority and high interest in my research because it was identified in two independent database searches. Overall, I have had success in running RACE reactions on the four candidates. The bands that appeared on the final gels for these four candidates are mostly distinct, with some that are smeary. This means that there is a product from the RACE reactions and that I should be able to get some sequences. However, I have had little success in obtaining sequences from the RACE products, and thus have made no progress on the database search. Future possibilities, and recently started, include sequencing the RACE products with aid from the TOPO protocol, which can hopefully allow successful sequencing.

Professor Hadley Horch
References
