Singing-Dependent Gene Expression in Zebra Finch Brains

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Because zebra finches, like humans, are vocal learners, they represent an ideal model for investigating the neural basis of language acquisition, as well as any complex, learned behavior. Singing in these birds is associated with increased activity in discrete brain regions called vocal nuclei. Four major vocal nuclei (HVC, LMAN, RA, and Area X) make up two neural pathways essential for both singing and song learning. One way to investigate how this system functions is to study gene expression. A recent experiment used microarrays to detect mRNA of thousands of different genes in zebra finch brain tissue.

My research goal for this summer was to use in situ hybridization (ISH) to further characterize expression of individual genes detected by the microarrays. ISH uses probes that bind to mRNA to localize expression of particular genes in brain tissue. The genes I studied displayed robust expression patterns in the microarray; many also have known specific functions. I ordered bacterial stocks containing plasmids with zebra finch cDNA clones. For each gene, I grew the bacteria, extracted the plasmid, and then PCR amplified and gel purified the gene of interest. I used the amplified cDNA clone and T3 RNA polymerase to synthesize $^{35}$S-labeled RNA probes. I then hybridized the probe to zebra finch brain tissue, where it bound to complementary mRNA sequences.

As a positive control, I first performed ISH to detect expression of EGR1, a transcription factor known to be increasingly expressed in the four major vocal nuclei after a bird sings for 10-30 minutes. The results of this experiment confirmed that my technique was reliable. I then performed ISH for eleven other genes, using brains from birds that had either been silent when sacrificed or had been singing for different amounts of time, depending on the pattern demonstrated in the microarray study. A preliminary analysis shows three genes (THBS2, HSPH1, and SS18L1) that have distinct patterns of expression in the zebra finch vocal nuclei. THBS2 (thrombospondin-2) codes for a protein involved in synapse development. My experiment confirmed the expression pattern found in the microarray—increased expression in RA compared to other vocal nuclei, as well as the rest of the brain. This gene may be involved in the maintenance of the cytoskeletal structure of RA, a particularly important brain region that is responsible for the song system’s motor output. For HSPH1 (a heat shock protein), the microarray showed a pattern of increased expression in LMAN, RA, and HVC in singing birds compared to silent birds. My experiment showed increased expression in the entire brain region containing these nuclei, especially in LMAN and HVC. The singing bird’s brain also displayed increased expression in Area X compared to the surrounding region; the microarray did not detect this, perhaps because the striatal region where Area X is found showed high expression in silent animals. SS18L1 is involved in dendritic growth. The microarray showed a steady decrease in SS18L1 expression in all four nuclei over seven hours of singing. My results confirmed this pattern, with the largest decrease in Area X; expression was relatively low throughout the singing bird’s brain.

Of the genes that did not display expected expression patterns, some point to one limitation of the microarray, in that it only detected expression in the vocal nuclei and not in the rest of the brain. For example, the microarray showed a singing-related decrease in LMAN expression of an unknown gene 0061P0031E03. My experiment showed decreased expression of this gene in the singing bird’s entire brain, so this expression may not be strictly associated with the song control system. Further, for all of the genes examined, the cDNA clone sequences and the PCR product sizes need to be confirmed. Future experimentation will address these issues.

Faculty Mentor: Osceola Whitney
Funded by the Howard Hughes Medical Institute Summer Fellowship