Singing-dependent gene expression in the zebra finch brain
Michelle Lawson, 2012

My summer research focused on identifying genes that are differentially expressed in the brains of zebra finch songbirds. Zebra finches and humans are among the few species that exhibit vocal learning, thus study of zebra finches may have implications for similarly complex behaviors, including spoken language.

Four major interconnected regions in the songbird brain control singing. The cortical region LMAN and striatal region Area X are necessary to learn song, while the cortical regions HVC and RA are necessary for adult songbirds to sing. In all vocal learning birds, including songbirds, singing induces gene expression within these or similar major song control brain regions (Jarvis and Nottebohm, 1997; Kimpo and Doupe, 1997; Jarvis et al., 2000; Mello and Jarvis, 2000). More recently, networks of singing-dependent genes were identified in the zebra finch brain. For this, they used a high-throughput technology called a microarray to look at thousands of genes at once. They found that a large proportion of genes (~5,000) are differentially expressed across multiple brain regions before singing, and furthermore, that a remarkable ~10% of the coding and non-coding genome is regulated by singing (Whitney et al. in prep).

My project’s goal was to use in situ hybridization (ISH) to further characterize the expression of a small set of interesting genes identified in the microarray data, such as transcription factors and intracellular signaling regulators. My first challenge was to identify genes with microarray data that was consistent within a group (either a brain region or a time point) but varied between groups (either between regions or between time points). I found several genes for further ISH analysis, including IQSEC3 and MEF2. I then performed ISH on brains of silent and singing zebra finches using radioactive 35S-ribonucleotide probes. For this, cryosections of zebra finch brain tissue was mounted onto glass slides. Radioactively labeled ribonucleotide probe was hybridized overnight to complementary mRNA sequences in the tissue that corresponded to the gene of interest. mRNA expression was visualized by exposing the hybridized brain sections to autoradiographic film. A higher signal on the film indicated a region of higher gene expression.

I first examined the EGR1 gene as a positive control to ensure I performed the in situ protocol correctly. The EGR1 probe I tested exhibited a pattern of expression identical to that of previous work (Jarvis and Nottebohm, 1997). I then tested the expression of IQSEC3, a neuron-specific gene that is part of the post-synaptic density (PSD), a specialized protein complex for the transduction and modulation of signaling between neurons. IQSEC showed uniquely high expression in HVC relative to the surrounding brain regions and other song-control regions. Next, I will be testing the expression of MEF2, a transcription factor involved in cellular differentiation and development. I expect MEF2 to be highly expressed in HVC and LMAN, but not in Area X, RA, or surrounding tissue. MEF2 is an intriguing molecule to study, as its role in cellular differentiation and development indicate it might be involved in song acquisition.

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