Genetic Analysis of Anxiety Related Behaviors by Gene Chip and *In situ* Hybridization of the Hippocampus and Amygdala of C57BL/6J and AJ Mice Brains
INTRODUCTION

To study the relationship between an animal's behavior, the brain systems involved, and the underlying genetic make-up, a researcher must move from the behavior and gross anatomy down to the molecular level. Initial tests performed by researchers Chantal Mathis, Steven Paul, Jacqueline Crawley, and Howard Gershenfeld have characterized the inbred strains of A/J and C57BL/6J mice as being on the extremes of an emotional behavioral continuum. Tests with A/J and C57BL/6J mice show the levels of exploratory behavior in light/dark models are much higher for C57BL/6J mice than for A/J mice. Exploratory activity in an open field also shows C57BL/6J as having significantly higher levels of exploratory behavior than A/J. Passive avoidance performance show no evidence of acquisition of passive avoidance task at low levels of footshock in C57BL/6J mice, yet A/J mice readily acquire memory task at low levels of footshock.
C57BL/6J mice are
- less "emotional" and "behaviorally inhibited"
- more active
- less sensitive to novelty
  than A/J mice.

These characteristics allow one to characterize A/J mice as inhibited and C57BL/6J mice as less inhibited. These significant differences between the phenotypes of these two inbred stains gave indirect evidence that genes were contributing to the variation in phenotype and gene mapping could then be conducted for quantitative trait loci (QTL) analysis.
METHODS

Traditional methods in molecular biology generally work on a "one gene in one experiment" basis, which means that the throughput is very limited and the "whole picture" of gene function is hard to obtain. In the past several years, a new technology, called a "GeneChip", DNA microarray or microarray assay has been developed. Researchers have used GeneChip probe arrays to study the regulation of gene expression associated with a wide variety of basic biological functions, including development, hormonal signaling, and circadian rhythms. An experiment with a single GeneChip can provide information on thousands of genes simultaneously - a dramatic increase in throughput. This technology monitors the whole genome on a single chip so a better picture of the simultaneous interactions among thousands of genes is seen and determinations of expression level (abundance) of genes can be made.
Gene Chip

Relative size of Gene Chip

Pictures modified from GeneDetect.com
GeneChip Analysis of Hippocampus from A/J and C57BL/6J Mice

The Gershenfeld Lab used Gene Chips to determine which genes are activated and which genes are repressed when comparing the hippocampus of A/J and C57BL/6J mice.

1. Obtain the cells of the hippocampus of A/J and C57BL/6J mice. Extract the mRNA because DNA microarray analysis is based on the assumption that transcriptions level of genes are equal to the mRNA present in cell.
2. Since mRNA degrades so easily, make cDNA from mRNA using reverse transcription. Degrade the remaining mRNA.
3. Label cDNA of the A/J samples and C57BL/6J samples with flourescent labels.
4. Each spot on gene chip slide is made of DNA, (actually a 25 mer oligonucleotide) that can base pair w/ cDNA.
5. Hybridize cDNA samples to chip and incubate. Each chip covers 7000 genes. Base-pairing (i.e., A-T and G-C for DNA; A-U and G-C for RNA) or hybridization is the underlining principle of DNA microarray.
6. Wash unbound cDNA off. All that is left is what has specifically bound to the DNA spots.
7. Scan the chip with laser to detect the bound cDNA. Images from the A/J and C57BL/6J chips are stored in computer for later signal processing and comparisons. Sophisticated software algorithms compare expression levels of each gene and calculate the extent (fold) increases, decreases, or no significant changes.
In Situ Analysis of Hippocampus from A/J and C57BL/6J Mice

In order to validate the GeneChip data obtained and to find the patterns of genes and their expression levels in the hippocampus and amygdala of the brains of the A/J and C57BL/6J mice, in situ hybridization will be used. In situ hybridization allows a particular mRNA species to be localized in its normal anatomical environment (i.e., tissue section.)

1. Since the data on the GeneChip experiment suggests that genes RGS5, YWHAQ, and EBAF are those involved in "anxiety behavior," 45 bp oligonucleotides of complimentary sequences to genes RGS5, YWHAQ, and EBAF are obtained.
2. The oligo probes will be labeled radioactively by incorporating about 16 bp worth of 35 Sulphur onto them.
3. These labeled oligos will be hybridized to slides of 14um sections of hippocampus and amygdala tissue sections from A/J and C57BL/6J mice. Unbound oligos will be washed off to avoid background.
4. Visualization of the gene expression is done by exposing the slides to photographic film for 4-7 days, and then developing the film.
Brain cross section showing fluorescently labeled probe
(modified from www.affymetrix.com)
RESULTS

GeneChip analysis show increased expressions of genes RGS5, YWHAQ, and EBAF. This suggests that these are the genes involved in "anxiety behavior" in A/J and C57BL/6J mice. *In situ* hybridization will be performed to compare the level and/or location difference of RGS 5, YWHAQ, and EBAF expression in the brain regions of A/J and C57BL/6J mice. Ideally, there will be more RGS 5, YWHAQ, and EBAF expressed in the hypothalamus of C57BL/6J than that in A/J. Real time PCR will be used to further validate the potential difference.
in Situ hybridization showing mice hippocampus
(modified from www.unifr.ch/biochem/ABRECHT/LECTURES/InSitu)
*in Situ* Hybridization showing mice hippocampus
(modified from www.unifr.ch/biochem/ABRECHT/LECTURES/InSitu)
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