Progress Report for Moran Foundation Funded Work

Principal Investigator: **Graeme Mardon, Ph.D.**

Project Title: **A Biochemical Screen for Genes Required for Mammalian Brain Development (Revised)**

Project Year: **1999-2000**

Project Number: **99-0103**

Summary of Progress

The overall goal of this project is to look for proteins that physically interact with the Dach proteins. Since *Drosophila* Dac is known to bind other proteins and form complexes that act as transcriptional regulators, we believe that the mammalian Dach proteins will similarly bind other, as yet unidentified partners, during development. We initially suspected that *Dach1* was required for normal lung development but have since concluded that the cause of death in mouse *Dach1* mutants is more likely to be a defect in brain development. Therefore, we have changed the emphasis of this proposal to the brain, both in flies and mice (*dac* is also required for normal brain development in *Drosophila*). We will use the yeast two-hybrid CytoTrap© System (Stratagene) to find such proteins. These proteins are likely to be important regulators of brain development. The CytoTrap© yeast two-hybrid screen allows the physical interaction of two proteins to be used as an in vivo selection for the growth of yeast. We proposed to use the conserved domains of the Dach protein as "bait" to identify binding partners expressed from an embryonic brain cDNA library (the "target"). Primary analysis of candidate binding proteins will be carried out in four ways. First, putative binding partners will be scrutinized by of series of genetic tests to remove likely "false positives" from the screen. Second, binding of candidate partners will be verified using co-immunoprecipitation and in vitro biochemistry. Third, cDNAs encoding true Dach-binding partners will be sequenced to determine the identities of the genes. Fourth, the expression patterns of novel genes or known genes with no previously characterized function during development will be studied. We have made good progress toward building the constructs needed to conduct these studies. These experiments will provide a powerful means to identify a whole new set of genes that are likely to play important roles in human brain development and that are thus candidate disease loci.

This project is still in progress.

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