

436c Genomic Analysis of Hepatic Metabolite Pools

R. Michael Raab, Matthew S. Wong, and Gregory Stephanopoulos

Type II Diabetes mellitus is a genetically complex disease characterized by insulin resistance, which results in simultaneous hyperglycemia and hyperinsulinemia. As the incidence of type II diabetes grows throughout the world, more research groups are investigating the problem, however traditional mapping techniques have not been successful in explaining the underlying genetic components of the disease. Despite these previous efforts, high-through-put experimental techniques provide new approaches to studying disease pathogenesis and regulation in pertinent molecular pathways.

Hepatocytes are particularly important to maintaining whole body glycemia as a source of glucose and other substrates during non-feeding periods. Our work has focused on discovering new genes that influence hepatic metabolism. In our investigations we quantify hepatic metabolism in terms of metabolite pool distributions that describe flux rates within relevant metabolic pathways. Using DNA microarrays containing 17,000 unique gene probes, we have monitored hepatic gene transcription under normal, insulin resistant, and fasting states in C57 mice. From this data set we have identified 41 different genes that are highly discriminatory of the treatment groups.

To characterize genes identified by our analysis, we have developed combinatorial RNA-interference (RNAi) based gene silencing techniques for primary hepatocytes. Using RNAi we screened genes that were over-expressed within the set to study loss of function effects on the size of key metabolite pools. We analyzed the results using a variety of multivariate methods to determine which genes had a predominant affect on the metabolite distributions. Taken together, by combining DNA microarrays, RNAi based gene silencing, and intracellular metabolite measurements with advanced data analyses, we seek to identify novel regulatory processes affecting hepatic insulin resistance.