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DNA microarray-based profiling of global gene-expression changes in myotonic dystrophy

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Myotonic dystrophy (DM) is an autosomal dominant neuromuscular disease with highly variable multisystemic manifestations. The mutation underlying DM is an unstable (CTG)n expansion in the 3´ UTR of the myotonic dystrophy protein kinase gene (*DMPK*). The pathophysiological mechanism(s) of the expanded (CTG)n repeat remains unclear. Various effects have been proposed, most recently a gain of function for mutant *DMPK* transcripts that results in a generalized RNA metabolism defect, mediated through one or several *trans*-acting proteins involved in RNA processing. This would in turn lead to the loss of function of multiple genes by qualitatively or quantitatively affecting post-transcriptional RNA processing, splicing or nuclear export of their transcripts. To test these hypotheses, we examined global mRNA expression changes between DM patients and normal controls by comprehensively and simultaneously profiling more than 6,800 human genes with oligo-based Genechip microarrays (Affymetrix). Total, nuclear and cytoplasmic RNA fractions of DM patient lymphoblastoid cell lines (four adult-onset, one congenital) as well as primary undifferentiated myoblasts and differentiated myotubes (one adult-onset, one congenital) were profiled. DM myoblasts in culture showed a reduced differentiation rate to myotubes and a tendency to dedifferentiate, suggesting a general block in or reprogramming of differentiation. Expression profiles of DM cell lines differed considerably from controls. Between the different DM cell lines profiled, many of the more than 6,800 genes assayed showed dysregulation. Moreover, comparison of nuclear and cytoplasmic fractions suggested a defect in the nuclear export of some processed transcripts. The number of genes dysregulated and the degree of dysregulation correlated with expansion size. Functions of the dysregulated genes were highly varied. In conclusion, DNA microarray expression profiling identified several novel DM phenotype effector candidate genes that may explain the complex pathogenesis of DM.

LaBrie, Samuel

Arabidopsis thaliana gene expression microarray services

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Incyte has developed a new gene expression microarray (GEM) for analysis of the plant functional genomic system *Arabidopsis thaliana*. This GEM features over 7,000 array elements from a non-redundant cDNA collection; nearly 30% of the estimated 25,000 genes in this organism. Over 2,000 of these clones represent genes that have functional annotation in public databases. We are offering hybridization and analysis services for the *Arabidopsis* GEM via Incyte and Genome Systems. *Arabidopsis* is the primary model system for the study of dicot plant genomics. Academic and commercial groups are using the combination of genomic and genetic tools available for *Arabidopsis* to study complex problems in plant biology. We have performed a few biological studies using the *Arabidopsis* GEM to validate the platform and provide examples of possible applications. We will present sample data on these studies.

Landgraf, Jeff R.

Gene expression profiling in Arabidopsis using DNA microarrays

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The microarray facility at MSU is part of the Arabidopsis Functional Genomics Consortium (AFGC), a collaboration recently funded by the National Science Foundation. Together with Shauna Sommerville at Carnegie Institute and Mike Cherry at Stanford University our goal is to make DNA microarray technology available to the academic community through the WWW. The first year of our project will be devoted to optimisation of the technology and preparation of the microarrays containing thousands of *Arabidopsis* cDNAs. The DNA fragments spotted on the microarray will be amplified from the collection of cDNA clones sequenced by Tom Newman. By hybridising these arrays with probes corresponding to mRNA extracted from different tissues and organs or from plants subjected to different stimuli, global gene expression patterns can be investigated. We will also examine the expression of plant specific genes of unknown function under a set of standardised conditions. The microarray facility is scheduled to begin providing service to academic researchers in year two of our project and the service will be continued in year three. Other investigators in the AFGC will establish a gene knockout facility. Together these technologies should provide the scientific community with a set of powerful tools that can be used in their efforts to understand the function and interrelationships of the genes of *Arabidopsis* and other plants.

Lee, Sang Y.

Development of DNA microarray for the monitoring of Escherichia coli metabolism

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We developed a microarrayer system for the manufacture of DNA chips. The 3-axis robot was designed to automatically collect samples from 96- or 384-well microtiter plates using up to 16 pens simultaneously and deposit them on a modified glass slides. This is followed by a wash/dry operation and the cycle is repeated with a new set of samples. This system can deposit cDNA with an interval of 150 micrometer to 80 micrometer in size, thus allowing high-density DNA chips of about 5,000 ea/cm^2 to be made. An in-house Visual C++ program running on a Pentium computer controls the whole procedure. With our DNA chip microarrayer, we made DNA chips containing a set of genes whose products are involved in the primary metabolic pathways, heat shock response and protein secretion in *Escherichia coli*. Expression of these genes in *Escherichia coli* was monitored under various conditions: recombinant protein production, high-density cell culture, etc. Details of the step-by-step procedures for manufacturing DNA microarrayer and DNA chips will be presented. Also, the results from the expression monitoring of central *Escherichia coli* genes under various cultivation conditions will be presented.