Focused Metabolite Profiling for Dissecting Cellular and Molecular Processes of Living Organisms in Space Environments

2006 Center Director's Discretionary Fund Project

Water and Air Recovery/ Purification

Regulatory control in biological systems is exerted at all levels within the central dogma of biology: DNA→mRNA→$Enzyme_{inactive}$→$Enzyme_{active}$→Metabolites (Figure 1). Metabolites are the end products of all cellular regulatory processes and reflect the ultimate outcome of potential changes suggested by genomics and proteomics caused by an environmental stimulus or genetic modification. Following on the heels of genomics, transcriptomics, and proteomics, metabolomics has become an inevitable part of complete-system biology because none of the lower "-omics" alone provide direct information about how changes in mRNA or protein are coupled to changes in biological function. Analogous to the precedent "omics," metabolomics is the systematic study of collections of small molecules (i.e., metabolites) in a biological system (a cell, organ, or organism). In contrast to the traditional biochemistry approach in which specific metabolites and enzymes are studied, metabolomics takes a holistic view of the entire suite of metabolites (the metabolome) in an organism to capture the coordinated regulation of biological systems. Thus, metabolomics, coupled with other "omics" such as transcriptomics and proteomics, holds great promise for deciphering the functions of genes, predicting novel metabolic pathways, and providing insights to the regulation of a biological event, as well as for directing metabolic engineering of plants for human benefit. However, the challenges are much greater than those encountered in genomics because of the greater number of metabolites and the greater diversity of their chemical structures and properties. To meet these challenges, much developmental work is needed, including (1) methodologies for unbiased extraction of metabolites and subsequent quantification, (2) algorithms for systematic identification of metabolites, (3) expertise and competency in handling a large amount of information (data set), and (4) integration of metabolomics with other "omics" and data mining (implication of the information).

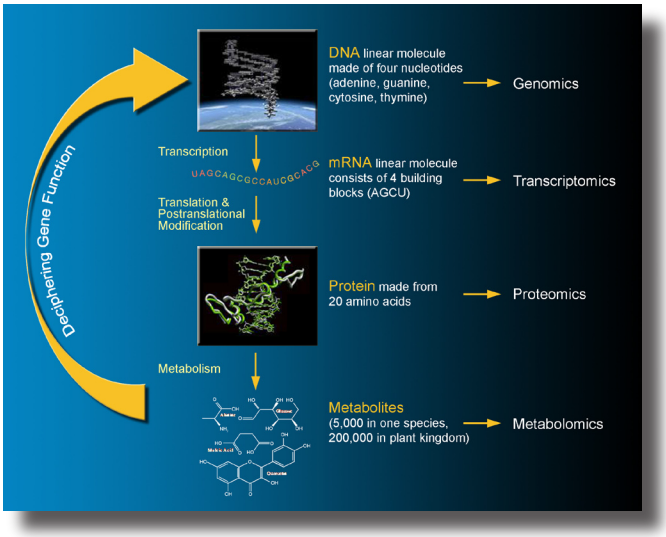

Figure 1. The central dogma of biology and the "omics" revolution.

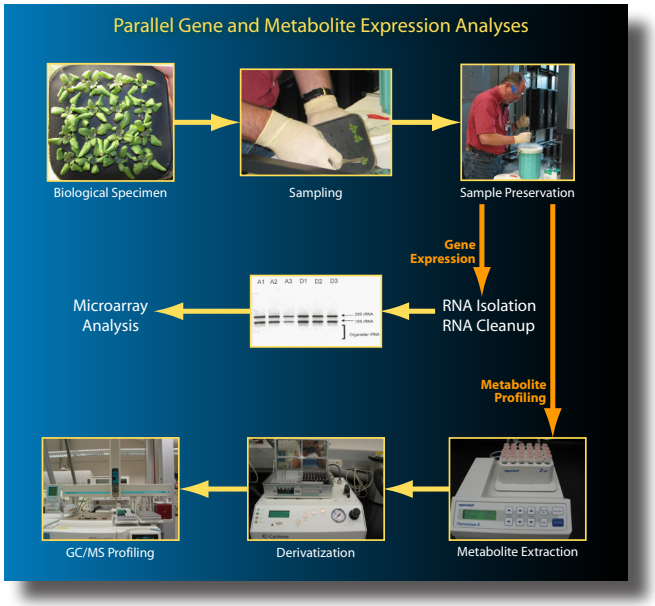

Figure 2. Analytical platform for metabolite profiling.

Project accomplishments include the following:

- A gas chromatography/mass spectrometry (GC/MS)-based analytical platform was developed and optimized (encompassing essential hardware [Figure 2]) for profiling small polar molecules. Procedures, software, and technical competency in metabolite profiling were also developed (also see "Development and Coupling of Metabolomics Capability With Transcriptomics To Dissect Cellular and Molecular Processes of Living Organisms" in *KSC Technology Development and Application 2005 Annual Report*).
- Metabolite profiling was completed for 54 samples generated in FY 2005 experiments.
- Microarray analysis for gene expression (about 22,000 transcripts) and data processing platform was completed.
- The Automated Mass Spectral Deconvolution and Identification System (AMDIS) was implemented and enabled to analyze the GC/MS raw data files for the purification of mixed spectra and the identification and quantification of target components (Figure 3). With the help of Bionetics, an Excel macro was developed to extract and format AMDIS outputs.
- A targeted mass spectra and retention index library was constructed for *Arabidopsis* metabolome and quantitative and qualitative information was extracted from the GC/MS metabolite profiles (70 unique mass tags are statistically responsive to carbon dioxide [CO_2] treatment).
- Competency was increased in statistics and bioinformatics to extract biological meaning from transcriptional and metabolite profiles, and work to interpret data continued in an effort to understand the differential effect of normally elevated CO_2 and superelevated CO_2 on plant growth and stomatal function.
- Four additional elevated-CO_2 experiments were conducted to obtain complementary information on transpiration (indicative of stomatal function), growth, and morphology.
- The occurrence in this plant model of previous observations from other plant species was confirmed, thus offering opportunities to use molecular tools to reveal the mechanism underlying the biphasic response of growth and stomatal function to increasing atmospheric CO_2 concentration.
- A manuscript focusing on stomatal function under normally elevated CO_2 and superelevated CO_2 was initiated.

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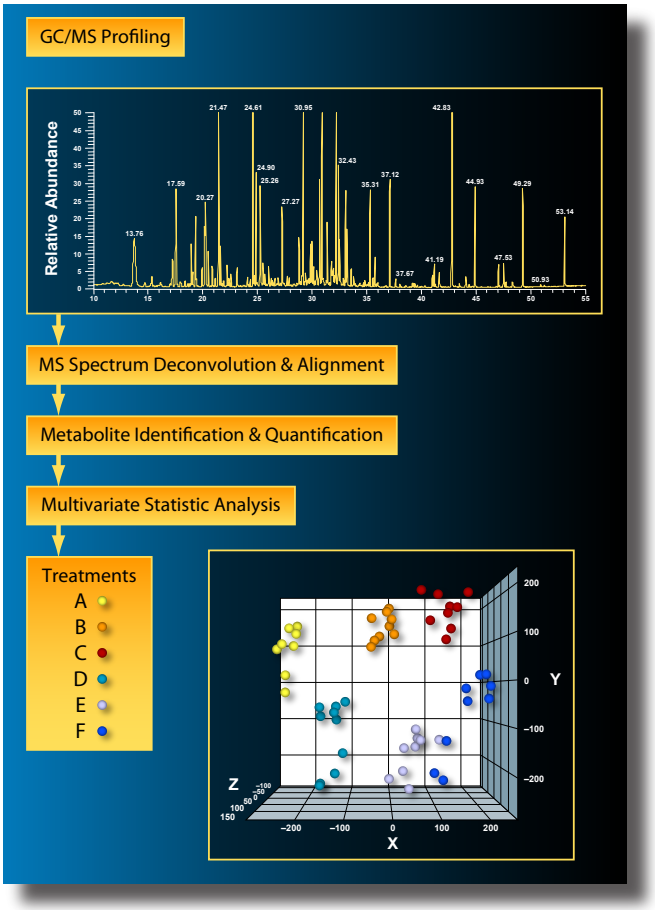

Figure 3. Data analysis and information extraction framework.