196f Molecular Approaches for Identification of Metabolic Engineering Targets for Enhanced Paclitaxel Accumulation

Susan C. Roberts, Nathan Ezekiel Nims, Camille Dubois, Nadia Boutaoui, Kham Vongpaseuth, and Elsbeth Walker

Metabolic engineering research has largely focused on microbes, with an emerging emphasis on plants and animals. Plants are critically important to the world's food resource, synthesis of bio-based goods (e.g., chemicals, energy, etc), and supply of pharmaceutically active products. Metabolic engineering of plant cell systems offers distinct challenges over microbial systems including slow plant growth, metabolic compartmentalization and redundancy, and lack of genetic characterization. There have been some recent successes in the reconstitution of plant biosynthetic pathways in fast-growing microbes, but because of the complexity of many plant products, transformation of entire pathways is often not possible. Therefore, the application of systems-level approaches to characterizing plant metabolism is necessary in the design of optimal plant-based systems, including those centered on plant cell culture.

Plant cell cultures represent a potential method for the large-scale production of important secondary metabolites. One significant example is the anti-cancer agent paclitaxel, a secondary metabolite produced by *Taxus*. Paclitaxel is a member of a family of related compounds known as taxanes. In *Taxus* suspension cultures, paclitaxel is often less than 10% of the total taxanes present. This low selectivity represents an excellent opportunity to increase overall accumulation through targeted metabolic engineering. We are focusing on the use of molecular approaches to delineate global metabolic control of paclitaxel accumulation in *Taxus* cell cultures. We are taking a two-pronged approach in identifying key regulatory genes. First, we have investigated expression of known pathway genes through Northern analysis, PCR and metabolite analysis via HPLC. Second, we have applied a novel systems-wide transcript profiling method to identify both up- and down-regulated genes upon induced paclitaxel accumulation. These genes may not be involved in paclitaxel biosynthesis, but rather transcriptional regulation, transport, secretion and degradation. This talk will highlight our research-to-date with an emphasis on novel approaches for profiling genetically uncharacterized systems.