

44. Defining Determinants and Dynamics of Cellulose Microfibril Biosynthesis, Assembly and Degradation

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Project Goals: The goals of this multidisciplinary project are to: (1) Establish platforms through reverse and forward genetics to identify and manipulate candidate genes that influence cellulose microfibril synthesis and structure; (2) Characterize the effects of altered candidate gene expression on cellulose microfibril synthesis and structure, and develop a mechanistic model for microfibril crystallization; (3) Determine the consequences of altering microfibril architecture on digestibility and integrate this information with nano-scale observations of enzymatic hydrolysis.

The central paradigm for converting plant biomass into soluble sugars for subsequent conversion to transportation fuels involves the enzymatic depolymerization of lignocellulosic plant cell walls by microbial enzymes. Despite decades of intensive research, this is still a relatively inefficient process, due largely to the recalcitrance and enormous complexity of the substrate. A major obstacle is still insufficient understanding of the detailed structure and biosynthesis of major wall components, including cellulose. For example, although cellulose is generally depicted as rigid, insoluble, uniformly crystalline microfibrils that are resistant to enzymatic degradation, the *in vivo* structures of plant cellulose microfibrils are surprisingly complex.

Crystallinity is frequently disrupted, for example by dislocations and areas containing chain ends, resulting in “amorphous” disordered regions. Importantly, microfibril structure and the relative proportions of crystalline and non-crystalline disordered surface regions vary substantially and yet the molecular mechanisms by which plants regulate microfibril crystallinity, and other aspects of microfibril architecture, are still entirely unknown. This obviously has a profound effect on susceptibility to enzymatic hydrolysis and so this is a critical area of research in order to characterize and optimize cellulosic biomass degradation.

The entire field of cell wall assembly, as distinct from polysaccharide biosynthesis, and the degree to which they are coupled, are relatively unexplored, despite the great potential for major advances in addressing the hurdle of biomass recalcitrance. Our overarching hypothesis is that identification of the molecular machinery that determine microfibril polymerization, deposition and structure will allow the design of more effective degradative systems, and the generation of cellulosic materials with enhanced and predictable bioconversion characteristics.

We believe that the most effective way to address this long standing and highly complex question is to adopt a broad ‘systems approach’. Accordingly, we have assembled a multi-disciplinary collaborative team with collective expertise in plant biology and molecular genetics, polymer structure and chemistry, enzyme biochemistry and biochemical engineering. Our team is using a spectrum of cutting edge technologies, including plant functional genomics, chemical genetics, live cell imaging, advanced microscopy, high energy X-ray spectroscopy and nanotechnology, to study the molecular determinants of cellulose microfibril structure.

Specifically we are coupling with an analytical pipeline to characterize the effects of altering microfibril architecture on bioconversion potential, with the goal of generating predictive models to help guide the

identification, development and implementation of new feedstocks. We are using *Arabidopsis thaliana* and *Brachypodium distachyon* as model dicotyledon and grass species, respectively.

We have established an EMS-mutagenized *B. distachyon* Bd21-3 population of approximately 5000 M2 families. By integrating TILLING methods with Illumina sequencing of target gene amplicons, we have developed an informatics protocol for efficiently identifying single base pair mutations. To date, we have screened for mutations in six genes of interest. A total of 67 SNPs were detected and 55 SNPs were confirmed through Sanger sequencing in 6 target regions. Our results to date indicate that the *B. distachyon* mutant population and the TILLING by sequencing protocol have great potential for functional studies and should greatly facilitate reverse genetic approaches for gene discovery in this model grass system.

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