BIOENGINEERING FIBER QUALITY: MOLECULAR DETERMINANTS OF FIBER LENGTH AND STRENGTH

Thea A. Wilkins
Assistant Professor
University of California
Davis, CA

Abstract

In order to dissect the molecular mechanism of fiber development, and hence, fiber quality, our efforts continue to focus on investigating the regulation of genes with a pivotal role in turgor-driven cell expansion. Ultrastructural data and molecular studies characterizing molecular and cellular events associated with fiber development revealed that fiber expansion encompasses four distinct substages. The parallel between that rate of fiber expansion and expression of vacuolar H^+-ATPase subunits identifies expression patterns of "expansion" genes in developing cotton fibers. Future efforts will be directed towards the identification and isolation of the regulatory factor(s) that activate the V-ATPase and other expansion-related genes as a means to manipulate the rate and/or duration of expansion in developing cotton fibers to enhance fiber quality via genetic engineering.

Introduction

Many important fiber quality parameters are determined by genetic and environmental factors that control the rate and duration of fiber expansion, and hence, the size and shape of the mature fiber. Fiber expansion, in turn, is a complex biological process that entails the coordinate regulation of cell wall biosynthesis and maintenance of cell turgor. While developmental signals control the duration of trichome expansion, the rate of cell expansion is dicated by the interactions of non-cellulosic polysaccharide polymers with cellulose microfibrils in the cell wall (Carpita and Gibeaut, 1993). In fact, the rate-limiting step to cell expansion is believed to be linked to the degree of uncoupling between xyloglucans and cellulose microfibrils. As the structure and rigidity of the cell wall is relaxed during cell expansion, via loosening of the connections between the non-cellulosic matrices and cellulose microfibrils, cell turgor forces the fibrillar material to separate, thereby allowing the deposition of newly synthesized cell wall components.

In order to develop germplasm with improved fiber quality using molecular strategies, it is essential to identify the genetic factors that control fiber development and therefore directly impact fiber quality. One approach selected by a number of cotton groups to address this question involves the characterization of fiber-specific genes isolated by differential screening of cDNA libraries or a PCR-based method called differential display. Several cotton genes have been isolated in this fashion, although the function remains unknown for many of them, and for others, there is no evidence that supports an important role for fiber-specific genes in fiber development or fiber quality. While there is no doubt that the isolation of fiber-specific genes is potentially important to bioengineering fiber quality in the long-term, this approach has been quite disappointing in the short-term.

Our approach to unraveling the genetic mechansims regulating fiber quality has focussed initially on developing a thorough understanding of fiber biology at the molecular and cellular levels. Coupled with molecular analyses, structural studies of developing cotton fibers have fortuitously provided a holistic perspective on the dynamics of fiber expansion. Since expansion in developing cotton fibers is highly accentuated relative to other diffuse-growing cell-types (Tiwari and Wilkins, 1995), genes important to cell expansion, but not to cotton fibers *per se*, have been targeted for the characterization of molecular events associated with fiber expansion.

Materials and Methods

Plant material was obtained from *Gossypium hirsutum* L. cv. Acala SJ-2 maintained under greenhouse conditions with presribed temperature regimes as described in Wan et al. (submitted). For structural studies and immunocytochemistry, ovules were prepared by the method of rapid freeze-fixation and freeze-substitution for optimal preservation of cellular ultrastructure according to Tiwari and Wilkins (submitted). Enzyme assays and molecular experiments were performed using standard published procedures as outlined in Wan et al. (submitted).

Results and Discussion

The vacuole is a multifuctional subcellular compartment that plays a key role in osmoregulation and the maintenance of cell turgor, the driving force of fiber expansion. Dynamic changes in the number and molecular weight of tonoplast proteins in expanding cotton fibers indicate an increase in the channels, pores and transporters necessary to support rapid, prolonged expansion of developing cotton fibers (Wan et al., submitted). The vacuolar H^+-ATPase (V-ATPase), one of two electrogenic proton pumps located in the tonoplast, provides the requisite energy to mediate the influx of ions, metabolites and osmoregulatory solutes requiring active transport into the vacuole. Cotton clones encoding the major subunits of the V-ATPase holoenzyme (Wilkins, 1993; Wan and Wilkins, 1994; Hasenfratz et al., 1995) were utilized as molecular markers to monitor vacuolar activity during fiber development as an indirect measure of fiber expansion. The coordinate, spatial and temporal regulation of V-ATPase gene expression in developing cotton fibers parallels the

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1679-1680 (1996)
National Cotton Council, Memphis TN

rate of fiber expansion, measured as an increase in length expressed as mm per day. In light of the strong correlation between fiber expansion and V-ATPase gene expression, the V-ATPase mRNA pattern in developing fibers was used to define fiber "expansion" genes. Based upon the results of our efforts to date, we have successfully dissected fiber expansion into four discrete stages.

Four Stages of Cotton Fiber Expansion

Mobilization of cellular machinery (0-3 dpa): One of the many cellular events associated with the onset of fiber expansion, which commences on the day of anthesis (0 days postanthesis (dpa)), is the formation of an enlarged nucleus and nucleolus (Ramsey and Berlin, 1976). The enlargement of these organelles is presumed to presage a period of intense transcriptional activity, which necessitates an increase in the synthesis and assembly of ribosomes in the nucleolus in anticipation of a corresponding increase in translational activity. This period of fiber development is also characterized by a tremendous proliferation of membranes and organelle biogenesis needed to sustain cellular structures during the period of rapid polar elongation of developing fibers between 5 to 20 dpa. For instance, the biogenesis of the single, homogeneous large central vacuole by 2-3 dpa relies upon the fusion of preexisting vacuoles to some extent, but the incorporation of *de novo* synthesized provacuoles derived from the endoplasmic reticulum as well. Based upon immunolocalization of V-ATPase subunits, increased V-ATPase activity during early fiber expansion is initially associated with assembly of the active holoenzyme on the tonoplast of provacuoles (Tiwari and Wilkins, unpublished data). The change in the morpholgy and biochemical composition of the vacuolar membrane (tonoplast) denotes the functional differentiation of this organelle in support of turgor-driven cell expansion (Wan et al., submitted). In terms of cell wall components, the Golgi apparatus is reponsible for the synthesis, processing, assembly and transport of noncellulosic matrix polysaccharides and glycosylation of cell wall glycoproteins. Product-mapping of pectin and hemicellulose epitopes in developing fibers before and after the onset of expansion revealed that fiber expansion is accompanied by an increase in Golgi activity that involves 1) a proliferation of Golgi that increases the number of Golgi at least three-fold, as well as 2) an increase in Golgi efficiency via an increase in the number of cisternae per Golgi stack and associated *trans*-Golgi network (Tiwari and Wilkins, submitted). Thus, although fiber length has increased approximately 50-fold, it is quite clear that the fiber is still in a preparative stage of development that involves mobilization of the cellular machinery in anticipation of polar elongation of developing fibers.

Transition Period (3-5 dpa): It is during this stage of fiber development that the composition of the tonoplast changes dramatically to become "expansion-competent". The synthesis and incorporation of proteins into other compartments and subcellular organelles is also presumed to occur at this point, but what of the cell wall? Unlike the secondary cell wall of cotton fiber, the primary cell wall contains significant levels of pectins and hemicellulose. Pectins are noncellulosic polysaccharide polymers that form a matrix which interlocks the cellulose microfibrils and resists the longitudinal force generated by turgor. It is the dynamic interaction between cellulose and the noncellulosic matrices that dictates the ***rate*** of cell expansion. An important question in cell wall structure is the orientation of the pectin matrix relative to the cellulose-XG (xyloglucan) framework. During the formation of cotton fiber initials on the day of anthesis, the amorphous methyl-esterified pectin portion of the extracellular matrix undergoes a rapid conversion into a highly organized fibrillar network. Pectin fibrillar arrays organize into concentric rings that form a helical continuum from the base to the apex of the fiber. Immunofluorescence studies using the JIM-7 antibody revealed that this unusual fibrillar network of esterified pectins is oriented transverse to the axis of elongation in expanding fibers up to +3 dpa, but assumes a near-parallel orientation to the elongation axis by +5 dpa (Tiwari and Wilkins, unpublished data).

Polar Elongation of Developing Fibers (5-20 dpa): Between 5 to 20 dpa, the rate of fiber expansion increases dramatically to form a steep peak of expansion around 12-13 dpa, before rapidly declining to previous growth levels. At the peak of expansion, fiber length increases more than 2 mm per day. Interestingly, expression of osmoregulatory or turgor-related genes, especially vacuolar components such as the V-ATPase, are developmentally regulated in a manner that parallels the rate of fiber expansion, and are therefore termed "expansion" genes (Wan et al., submitted).

Termination of Fiber Expansion (20-25 dpa): Developmental signals trigger the onset of fiber expansion as early as 13-15 dpa, although the final phase of expansion terminates between 20 to 25 dpa. By this stage of fiber development, termination of fiber expansion is, in all probability, primarily due to a decrease in cell wall extensibility via the incorporation of cell wall structural proteins and the cross-linking of the primary cell wall components into a "fixed" structure. The first significant increase in fiber strength observed at this stage is consistent with the formation of a rigid primary cell wall. Thus, we believe that the data supports a model in which fiber length and fiber strength are actually two consecutive events in fiber development.

Given the complexities of biological processes involved in fiber expansion, altering or modulating the expression of any single gene in transgenic plants is unlikely to have any significant impact on fiber quality based upon the genes currently available for bioengineering, including fiber-specific genes. Clearly, the future challenge to cotton molecular geneticists is the identification of a global

regulatory switch that signals the onset of fiber expansion and is therefore responsible for regulating entire cascades of intricate biological processes that ultimately determine fiber quality. We believe that the identification of two cotton genes that are differentially expressed during fiber expansion in our laboratory provides a window of opportunity to take one step closer towards achieving this goal. In years to come, we hope to identify a *trans*-regulatory factor(s) that is responsible for the qualitative and quantitative regulation of expansion genes during fiber development.

References

Carpita, N.C. and D.M. Gibeaut. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. The Plant J. 3:1-30.

Hasenfratz, M.-P., C.-L. Tsou, and T.A. Wilkins. 1995. Expression of two related vacuolar H^+-ATPase 16-kilodalton proteolipid genes is differentially regulated in a tissue-specifc manner. Plant Physiol. 108:1395-1404.

Ramsey, J.C. and J.D. Berlin. 1976. Ultrastructure of early stages of cotton fiber differentiation. Bot. Gaz. 137:11-19.

Tiwari, S.C. and T.A. Wilkins. 1995. Cotton (*Gossypium hirsutum*) seed trichomes expand via diffuse growing mechanism. Can. J. Bot. 73:746-757.

Tiwari, S.C. and T.A. Wilkins. 1996. Immunolocalization of Cell Wall Polysaccharides Indicates that Maturation of the Golgi Apparatus is Associated with Increased Efficiency in Expanding Seed Trichomes of Cotton (*Gossypium hirsutum*). Submitted.

Wan, C.-Y., M.-P. Hasenfratz, D. Gunasekera and T.A. Wilkins. 1996. Functional differentiation of vacuoles in expanding cotton seed trichomes requires multiple levels of regulation that govern activity of vacuolar H^+-ATPases. Submitted.

Wan, C.-Y. and T.A. Wilkins. 1994. Isolation of multiple cDNAs encoding the vacuolar H^+-ATPase subunit B from developing cotton (*Gossypium hirsutum* L.) ovules. Plant Physiol. 106:394-395.

Wilkins, T.A. 1993. Vacuolar H^+-ATPase 69-kilodalton catalytic subunit cDNA from developing cotton (*Gossypium hirsutum*) ovules. Plant Physiology 102:679-680.