

TITLE: **Role of Phytohormone Signaling Pathways in Cotton Fiber Development**

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ABBREVIATIONS: Gh, *Gossypium hirsutum*; BR, brassinosteroid; BL, brassinolide; BRI1, Brassinosteroid Insensitive 1; LLR-RLK associated leucine-rich repeat receptor-like kinase; brz, brassinazole; XTH, xyloglucan endotransglycosylase/hydrolase; EXP, expansin; GA, gibberellic acid; SCF, SKP1 Cullin and an F-box protein; GID1, gibberellin insensitive dwarf; SLR1, slender rice

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ABSTRACT

Differentiation of cotton fibers involves sequential cell elongation and secondary cell wall deposition as they develop from fiber initials to highly elongated and thickened trichomes on the seed integument. Phytohormones are thought to play important regulatory roles in the development of these economically important cells. Previous experiments indicate that auxin, gibberellin and brassinosteroid promote cotton fiber development *in vitro*. To further evaluate the role of these phytohormones, we characterized genes that encode receptors and downstream components of their cellular signaling pathways. The function of these genes was confirmed by transgenic complementation of hormone insensitive mutants in model plants. In ongoing research, transgenic cotton plants that express transgenes designed to activate or suppress these phytohormone signaling pathways are being developed. Alterations in the characteristics of fiber produced by these plants will provide important clues about the role of these phytohormone signaling pathways in regulating the development of cotton fibers.

KEY WORDS:

cotton fiber development, phytohormone signal transduction, gibberellic acid, brassinosteroid,
cell elongation, secondary cell wall

Plants use various classes of hormones as signaling molecules to orchestrate a broad range of functions that coordinate their growth and development, all the while, maintaining a delicate balance with their environment. As these sessile organisms switch from one developmental program to the next, multiple hormones are able to affect one single cell type, while in other cases a single hormone can influence the growth and differentiation of different tissues in different ways (Chow and McCourt, 2006). Regardless of the breadth of reach of these hormones, these signaling molecules ultimately target specific transcriptional relays that modulate gene expression that is translated into what is recognized by our senses. In the context of the agronomically valuable crop, cotton, we have known for quite some time that hormones play an important and critical role in fiber development. This was first reported by Beasley and Ting (1973). However, the wide variety of secondary metabolites and the inaccessibility of plant genome sequences have made the identification and characterization of the action of these plant hormones difficult. Sequencing and molecular genetic characterization of several plant genomes, including *Arabidopsis* and rice, has provided a powerful new tool to identify and elucidate the mechanisms behind these signaling molecules. With the availability of extensive genomic and genetic data from model systems, along with the rapidly expanding genomic information from cotton that most often pertain to different stages of fiber development, we are now poised to develop a deeper understanding of how the developmental complexity of the fiber cell is influenced by these simple organic molecules. Therefore, we have initiated the quest to identify the signaling components of various hormones to understand their role in fiber development. Thus far, we have identified and characterized several components of the brassinosteroid (BR), gibberellic acid (GA) and auxin signal transduction pathways. Although it is possible that a non-Euclidian pattern of connectivity between different hormone signaling pathways could capacitate

the plant to produce the same output (i.e., fiber development) via different strategies, here we review the current models on BR and GA signaling and discuss how they have been used as a point of departure for future studies on hormone signaling in cotton fiber development.

RESULTS

Brassinosteroid signal transduction. Brassinosteroids are naturally occurring steroid-based hormones that elicit growth stimulation at nanomolar concentrations. Novel genetic screens of dwarf *Arabidopsis* mutants that are BR defective in biosynthesis and signaling have clearly demonstrated that BR is essential for the regulation of growth, photomorphogenesis, fertility and stress tolerance (Clouse and Sasse, 1998; Altmann, 1999). The biosynthetic pathway of BR proceeds toward brassinolide (BL), the most active form of these hormones, via two major routes, namely the early and late C-6 oxidation pathway (Choe, 2004; Szekeres and Bishop, 2006). Most of the BR biosynthetic genes have been characterized in *Arabidopsis* and in other plant species (Szekeres and Bishop, 2006). Like most hormones, BR homeostasis is maintained by BR-dependent transcriptional control of genes involved in BR metabolism. Studies have shown that steady state mRNA levels of biosynthetic genes are inversely proportional to the endogenous level of BR (Choe, 2004). This transcriptional feedback regulation is mainly due to a signaling cascade that begins with the perception of BL by the receptor, BRI1 (Brassinosteroid Insensitive 1). BRI1, is a plasma membrane-associated leucine-rich repeat receptor-like kinase (LRR-RLK) (Li and Chory, 1997) that engages other LLR-RLKs upon binding BRs at the cell surface, thereby initiating a complex intracellular transduction pathway that results in altered gene expression.

To better understand the role of BR in cotton fiber development, we used the cotton ovule culture system developed by Beasley and Ting (1973). Results of those experiments showed that

suppression of BR biosynthesis in cotton ovules by treatment with the BR biosynthesis inhibitor brassinazole (Brz) inhibits fiber formation, while addition of low concentrations of BL, in combination with GA and auxin, were found to promote fiber elongation (Sun et al., 2004; Sun et al., 2005). Furthermore, expression of fiber genes associated with cell elongation (xyloglucan endotransglycosylase/hydrolase *XTH* and expansin *EXP*) increased in ovules treated with BL and was suppressed by Brz treatment, establishing a correlation between brassinosteroid and fiber development. We identified and analyzed a cotton gene that encodes a BR receptor (*GhBR11*) orthologous to *BRI1* (Sun et al., 2004). The biological function of GhBR11 was confirmed by transgenic complementation of dwarf BR-insensitive *Arabidopsis* mutant plants homozygous for the weak allele *BRI1-5*, with the cotton gene. Ectopic expression of *GhBR11* resulted in recovery of the normal plant growth phenotype demonstrating that the *GhBR11* gene encodes a function BR receptor protein. Recently, transgenic cotton plants were developed that over-express or have suppressed expression of GhBR11 (Sun et al., 2007). Surprisingly, these plants produced fiber of nearly normal length but with altered secondary cell wall development. These results appear to indicate that BR is a primary regulatory factor controlling the maturation of cotton fibers *in planta*. Figure 1 shows a current model for BR action in cotton and fiber development.

Gibberellic acid signal transduction. GAs are a family of naturally occurring tetracyclic diterpenoids, some of which have intrinsic biological activities that affect many aspects of plant development. Although there are more than one hundred different GAs that have been fully characterized, only a few are known to have biological activity (Sponsel and Hedden, 2004). Bioactive GAs are known to regulate seed germination and storage mobilization, stem elongation, flower initiation, pollen and fruit growth, as well as root development (Thomas and Hedden, 2006) and GA is necessary for the development of fiber on

cultured ovules, indicating that GA plays a crucial role in fiber development. Similar to BR, GA also induces the expression of *XTH* and *EXP* in some plants species (Xu et al., 1996; Cho and Kende, 1997; Uozu et al., 2000). Advances in our understanding of how the GA-signal is transduced have been achieved primarily through the use of GA-deficient and GA-insensitive mutants. The most extensively studied component of the GA signaling pathway is the nuclear localized DELLA protein, which functions to repress GA-mediated growth (Peng et al., 1997; Silverstone et al., 1998; Dill et al., 2001; Ikeda et al., 2001; Gubler et al., 2002). Molecular genetic analyses have shown that DELLA proteins act as transcription regulators in GA-signal transduction. Exposure to GA de-represses gene expression through the degradation of DELLA proteins via the SCF E3 ubiquitin ligase/26S proteasome system (Thomas and Hedden, 2006). The recent, characterization of the rice GA insensitive mutant *gid1* led to the identification of the soluble GA receptor *GID1* (Ueguchi-Tanaka et al., 2005). *GID1* is a hormone-sensitive lipase-like protein that binds preferentially to bioactive GAs *in vitro* and interacts with the rice DELLA protein, *SLR1* (Ueguchi-Tanaka et al., 2005).

Recently, we have shown that the initiation of fiber elongation by the application of GA to cultured ovules also corresponds with increased expression of *XTH* and *EXP* (Aleman et al., 2007). In addition, expression of genes that encode for GA₂₀ oxidase and GA₃ oxidase, which catalyze the production of bioactive GAs, is suppressed by exogenous GA₃ while expression of the gene for GA₂ oxidase, which irreversibly metabolizes bioactive GAs to inactive forms, is up-regulated. These results agree with the feed-back model for GA biosynthesis recently proposed for *Arabidopsis*, maize and pea (Thomas and Hedden, 2006). To gain a better understanding on the GA signaling components in cotton, that lead to such changes in gene expression, two GA receptor genes (*GhGID1a* and *GhGID1b*) and two DELLA genes (*GhSLR1a* and *GhSLR1b*) that

are orthologs to the rice GA receptor (*GID1*) and the rice DELLA gene (*SLR1*), respectively, were characterized (Aleman et al., unpublished). Similar, to the GA biosynthetic genes, *GhGID1a*, *GhGID1b*, and *GhSLR1a* are under the negative feedback and feedforward regulation by GA. Recombinant GST-GhGID1s showed GA-binding activity in vitro that was augmented in the presence of GhSLR1a, GhSLR1b, or rice SLR1, indicating a complex formation between the receptors and the repressor proteins (DELLAs). This was further supported by the GA-dependent interaction of these proteins in yeast cells. Ectopic expression of the *GhGID1a* in the rice *gid1-3* mutant rescued the GA-insensitive dwarf phenotype, which demonstrates that it is a functional GA receptor (Aleman et al., 2007). These results have allowed us to choose the best gene candidates to introduce into cotton to modify GA signaling. The development of transgenic cotton over-expressing these genes is underway. Figure 2 shows a current model for GA action in cotton and fiber development. The altered GA response in these plants along with these preliminary results will give us insight to the role and regulation of the GA response pathway in relation to fiber development and enable us to identify genes responsible for the genetic diversity of fiber yield and quality traits.

DISCUSSION

Phytohormones play critical roles in the differentiation of cotton fibers. Analysis of the effects of exogenous hormones and hormone inhibitors on fiber development on cultured cotton ovules has provided a limited understanding of the role of these hormones in the regulation of fiber development. Recent advances in our understanding of the intracellular mechanisms that mediate cellular responses to phytohormones have come through extensive molecular genetic analyses in model plant systems. Application of this knowledge to cotton has begun to provide a more detailed understanding of the specific roles of individual phytohormone signaling

pathways in the regulation of cotton fiber gene expression and development. We anticipate that as this knowledge base grows, it will provide important new opportunities for the modification of cotton fiber development that may allow for optimized cotton fiber characteristics.

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FIGURE CAPTIONS

Figure 1. A model for BR action in cotton and fiber development. *GhBR11*, a cotton gene that encodes a plasma membrane-associated protein was shown to be a functional BR receptor (Sun et al., 2004). **No BR signal** (Left). The addition of Brz (a BR inhibitor), and the subsequent absence of BR, prevents GhBR11 from releasing the BR-signal. In cultured ovules, Brz, leads to the inhibition of fiber growth, which is correlated with the reduced expression of BR up-regulated genes involved in cell wall expansion (*EXP*, expansin; *TUB*, tubulin; *XTH*, xyloglucan endotransglycosylase/hydrolase, and *AGP*, arabinogalactan protein) (Sun et al., 2004; Sun et al., 2005). Fiber analysis shows altered secondary cell wall development between wild-type and *GhBR11* antisense plants in micrograph (top panel) and cross-sections (bottom panel) (Sun et al., 2007). **BR signal** (Right). In the presence of BL (active form of BR), the BR-signal is released by GhBR11. Addition of BL to cultured ovules leads to the up-regulation of genes involved in cell wall expansion and the production of fiber. Fiber analysis shows altered secondary cell wall development between wild-type and *GhBR11* over-expressing plants in micrograph (top panel) and cross-sections (bottom panel) (Sun et al., 2007). ? = unknown mechanism in cotton.

Figure 2. A model for GA action in cotton and fiber development. *GhGID1*, a cotton gene that encodes a soluble hormone-sensitive lipase like protein was shown to be a functional GA receptor that binds specifically to bioactive GA₄ (Aleman et al., 2007). **No GA signal** (Left). In the absence of GA, GhGID1 cannot bind to the cotton DELLA protein, GhSLR1, a negative regulator for the GA-signal. In cultured ovules this inhibits fiber growth which is correlated with a rapidly reduction of *GhSLR1*; *EXP*, expansin; *XTH*, xyloglucan endotransglycosylase/hydrolase, and *GA2ox* gene expression, and increases the expression of *GhGID1*, and the GA biosynthetic genes, *GA20ox*, and *GA3ox*. **GA signal** (Right). In the

presence of GA, GhGID1 binds GhSLR1, releasing the GA-signal. In cultured ovules, this promotes fiber development which is correlated with the rapid increased expression of cell wall expansion genes. Feedback regulation is shown by the up-regulation of the GA inactivating gene, *GA2ox*, and the reduced expression of the GA biosynthetic genes, *GA20ox* and *GA3ox* and *GhGIDs*. ? = unknown mechanism in cotton.

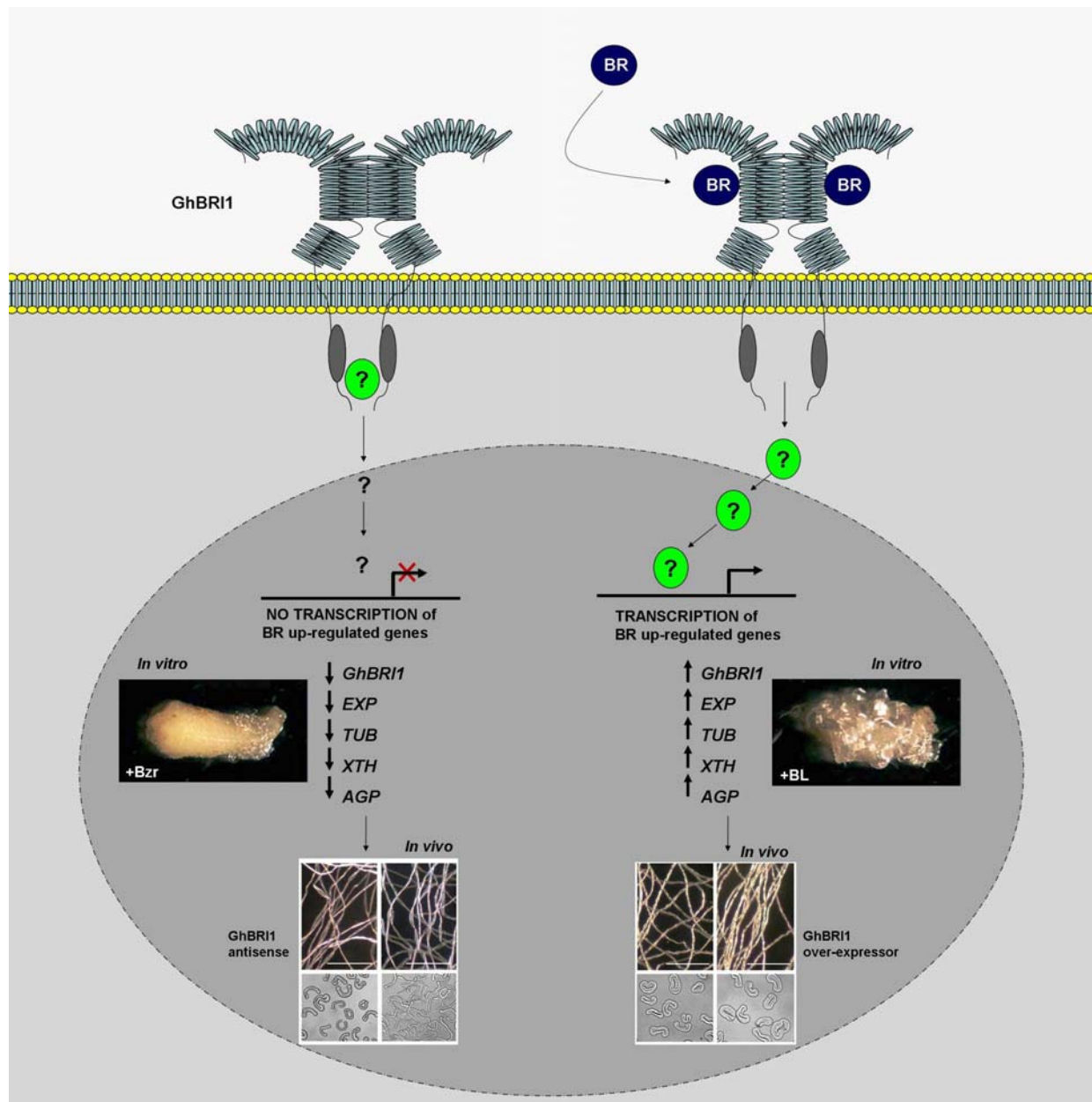


Figure 1.

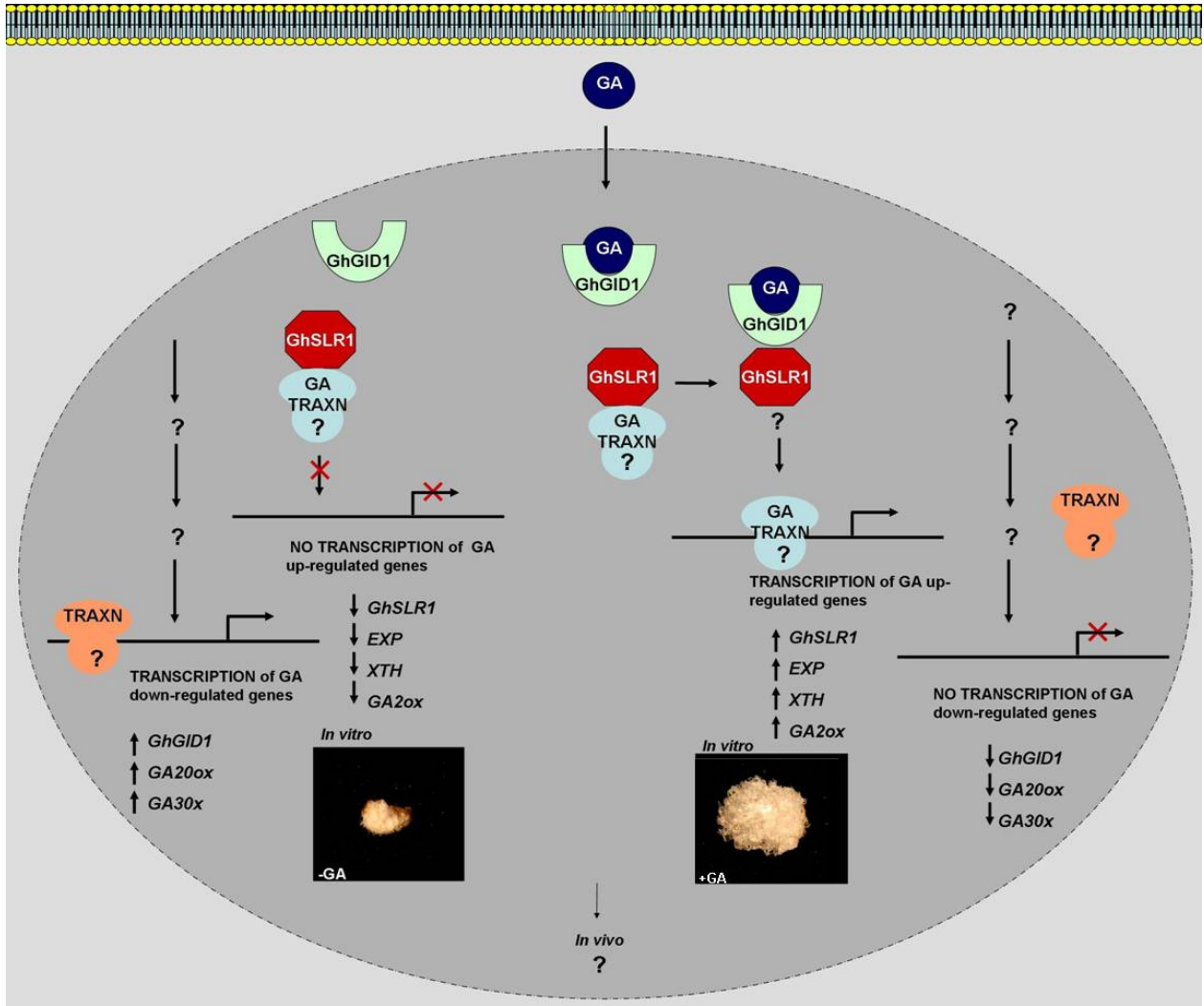


Figure 2.