



REVIEW PAPER

Cellulose biosynthesis inhibitors – a multifunctional toolbox

Mizuki Tateno*, Chad Brabham* and Seth DeBolt¹

¹ Department of Horticulture, University of Kentucky, 309 Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546-0312, USA

* These authors contributed equally to this paper

To whom correspondence should be addressed. Email: sdebo2@uky.edu

Received 5 August 2015; Revised 21 October 2015; Accepted 23 October 2015

Editor: Simon Turner, University of Manchester

Abstract

In the current review, we examine the growing number of existing Cellulose Biosynthesis Inhibitors (CBIs) and based on those that have been studied with live cell imaging we group their mechanism of action. Attention is paid to the use of CBIs as tools to ask fundamental questions about cellulose biosynthesis.

Key words: Cell wall, cellulose biosynthesis inhibitors, cellulose synthase, herbicides, microtubules.

Introduction

The primary cell wall is an elaborate matrix of polysaccharides interwoven among a relatively small amount of proteins and aromatic compounds (Vogel, 2008; Carpita, 2011). The interaction, rearrangement, and biochemical changes between these components give the cell wall its rigid, yet extensible architectural characteristic. The strongest element in the plant cell wall is a network of coalesced long linear chains of β -1, 4 linked glucose molecules, called cellulose microfibrils. Cellulose has evolved to serve as the structural reinforcement of the cell wall. As a chain of sugar units, cellulose displays a surprisingly high tensile strength (Somerville *et al.*, 2004). Cellulose microfibrils are organized in respect to the growth state of a cell. For example, in cells undergoing rapid expansion, microfibrils are often aligned perpendicular to the axis of growth (Baskin, 2005). Disruption of cellulose biosynthesis or alteration of microfibril alignment in the cell wall causes loss of directional cellular expansion, resulting in cells becoming radially swollen and growth organs becoming dwarfed. In this review, recent advances in our understanding of cellulose chemical perturbation and regulation by small molecules will be discussed. Chemicals that inhibit plant growth are globally referred to as herbicides, but are also referred to as drugs, small molecules or chemical inhibitors. For simplicity, we use the term herbicide or cellulose

biosynthesis inhibitor (CBI). Particular attention is paid to the use of advanced live cell imaging microscopy techniques and screening platforms for CBIs.

Cellulose biosynthesis in plants

It is important to convey to the reader that cellulose biosynthesis is complex, particularly when considering potential CBI targets. Therefore, we will briefly review the process of cellulose biosynthesis before focusing on CBIs.

Cellulose is synthesized at the plasma membrane (PM) by a multi-protein complex referred to as the cellulose synthase complex (CSC). CELLULOSE SYNTHASE A proteins (CESA) are the processive glycosyltransferases responsible for catalyzing the conversion of UDP-glucose to cellulose (Kimura *et al.*, 1999). The CSC is likely pre-assembled in the Golgi (Haigler and Brown, 1986) and transported to the PM via the trans-Golgi network and ultimately by cortical microtubule-assisted vesicle trafficking (Paredes *et al.*, 2006; Crowell *et al.*, 2009; Gutierrez *et al.*, 2009). A series of genetic experiments have shown that three different CESAs are needed to form a functional CSC (Taylor *et al.*, 2003; Desprez *et al.*, 2007; Persson *et al.*, 2007). Furthermore, freeze fracture electron microscopy images have revealed that the PM bound CSC

is a hexameric rosette-shaped complex (Saxena and Brown, 1997). It is believed that the CESA proteins in each subunit organize into a heterotrimeric complex (Desprez *et al.*, 2007) that possibly involves a stoichiometry of 1:1:1 between these three different CESA subunits (Gonneau *et al.*, 2014; Hill *et al.*, 2014). Rationalizing the number of active CESAs in a CSC has been guided by estimates of the numbers of glucan chains in a microfibril. However, this is still an area of debate and has been revised from a commonly cited 36 glucan chains in a microfibril to an estimate of 18 (Fernandes *et al.*, 2011) and more recently, 'at least' 24 (Wang and Hong, 2015).

Numerous accessory proteins are required for cellulose biosynthesis in plants, such as KORRIGAN, COBRA and CELLULOSE SYNTHASE INTERACTING protein 1 (CSI1/POM2), and CSI3. KORRIGAN, an endoglucanase (Roudier *et al.*, 2005), physically interacts with the CSC (Mansoori *et al.*, 2014; Vain *et al.*, 2014) and is thought to offer an editing role for the arising cellulose strands. COBRA, a glycosyl-phosphatidyl inositol-anchored protein, is also required for cellulose biosynthesis (Lane *et al.*, 2001). While COBRA's catalytic role remains unclear it has recently been found that it is critical to maintaining cellulose structure (Sorek *et al.*, 2014). Interestingly, KORRIGAN, COBRA, and CESA respond to CBI application in an analogous manner, suggesting they are cognate members of a CSC. In addition, the microtubule-CSC binding protein complex CSI-1/POM2 and CSI3, which is thought of as a molecular cross linker (Gu *et al.*, 2010; Bringmann *et al.*, 2012; Li *et al.*, 2014) was also found to interact with the CSC (Gu *et al.*, 2010; Bringmann *et al.*, 2012). Another such group of microtubule interacting accessory proteins that were recently described are the Companion of Cellulose synthase (CC) proteins (Endler *et al.*, 2015). The catalytic function for the CCs is still under investigation but it appears to be a marker for microtubule recovery from salt stress.

As it relates to the potential targets for a CBI, an interesting facet of the cellulose biosynthetic process is its complexity. Aside from the catalytic CESAs, each of these accessory proteins are plant specific and are valid targets for a CBI herbicide. Post-translational modifications such as phosphorylation have also been identified and found to influence cellulose biosynthesis (Chen *et al.*, 2010) and therefore it remains possible that targeted kinase inhibitors may induce a CBI-like mode of action.

Cellulose biosynthesis inhibitors

Group L herbicides have a mode of action that inhibits cell wall (cellulose) biosynthesis, as classified by the Herbicide Resistant Action Committee (HRAC), and are further subdivided by their structural chemistry. As chemical inhibitors of cellulose biosynthesis, cellulose biosynthesis inhibitors (CBIs) are useful for weed control in agriculture and are particularly used as pre-emergent herbicides in recreational lawns, golf courses, orchards, vineyards, and railroad tracks with a combined multi-billion dollar value. CBIs are of increasing importance in agriculture at present due to problematic rates of weed resistance to known herbicides and the development of resistance management strategies that involve multiple modes of action (MOAs). CBIs have no reported field resistance (Heap,

<http://www.weedscience.org>), which makes them attractive in such strategies. However, Arabidopsis mutants have been generated that confer resistance (or at least tolerance) to CBIs (e.g. Heim *et al.*, 1989; Harris *et al.*, 2012). It is not clear why field resistance is not more prevalent. One possibility is that these resistance loci were isolated from populations of intentionally mutagenized Arabidopsis seed and are associated with a fitness penalty (Harris *et al.*, 2009, 2012). While field resistance has not been observed to date, it appears quite possible that it could arise and a strategy of resistance management would be needed in any application regimen.

How to identify a CBI and its MOA

For a compound to be classified as a CBI, it must meet three criteria: 1) treated seedlings exhibit characteristic CBI symptomology of stunted growth and radial swelling in rapidly expanding tissue (Fig. 1A, B) where ectopic lignification is sometimes evident (as shown in Fig. 1A-inset in red); 2) reduced cellulose content in a dose-dependent manner; and 3) rapidly (<2h) inhibit the incorporation of ¹⁴C-glucose into the cellulose fraction of cell walls (Fig. 1C). As stated above, the complexity of cellulose biosynthesis makes it difficult to further elucidate the potential inhibitory mechanisms of CBIs. A considerable breakthrough in examining cellulose biosynthesis was achieved almost a decade ago with the functional complementation of the *procuste-cesa6* mutant with a translational fusion between YFP and CESA6, driven by its native promoter (Paredes *et al.*, 2006). This, along with advanced laser scanning (or spinning disc) confocal imaging systems, enabled the quantitative assessment of CESA behavior in living cells (Fig. 1D).

Live cell imaging of cellulose biosynthesis can also be applied to CBI MOA. Plants expressing the fluorescent protein reporter tagged CESA (CESA6, CESA3, and CESA5) are imaged within a 1–2h period after exposure to a CBI/herbicide at a saturating rate (Fig. 1D). It is therefore assumed that the disruption is a direct result of the MOA rather than a pleiotropic effect. Short duration movies (5–10 min in length comprising 60–100 frames) of live plants expressing YFP:CESA6 are generated and compared between CBI treated and untreated tissue (Paredes *et al.*, 2006; DeBolt *et al.*, 2007b). Using qualitative and quantitative assessments of behavior of the CESAs, the MOA were then characterized. Interestingly, but perhaps not surprising considering the multiple proteins involved in cellulose biosynthesis, different CBIs caused markedly different symptoms. To try and use this advanced imaging data to classify CBIs, we developed a categorization system based on how a given CBI disrupts the normal mobility and localization of fluorescently labeled CESA particles, both individually and in array (Brabham and DeBolt, 2012). The three different classification groups that have been proposed are CBIs that 1) cause clearance of CESA particles from the PM; 2) increase CESA accumulation in at PM accompanied by arrested (or slowed) CESA movement in the PM; and 3) induce modified CESA trajectory to PM and CESA speed at the PM focal plane. Recently, new CBIs have been discovered and characterized such as CESTRIN,

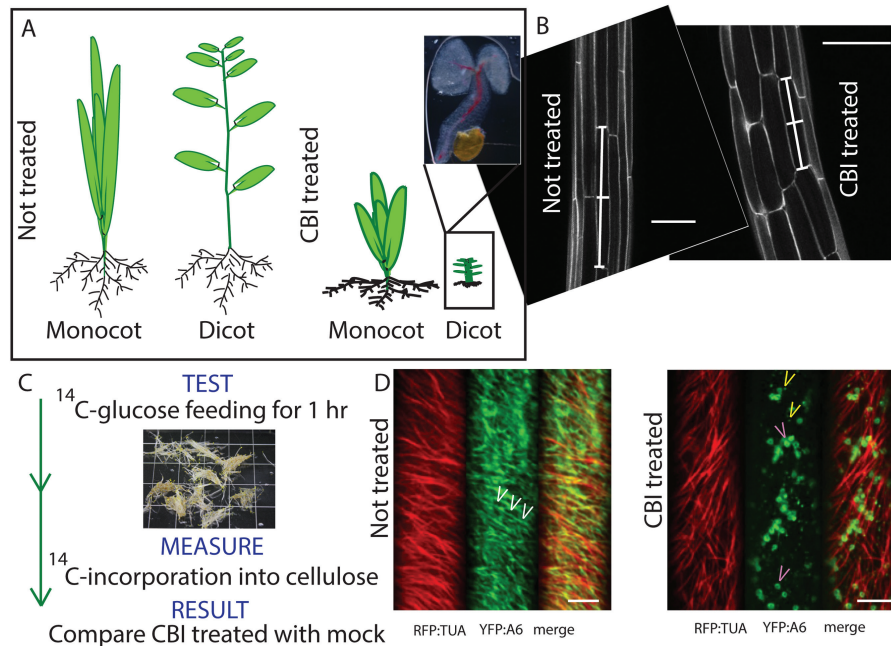


Fig. 1. CBI screening and characterization. (A) Representation of the effect of CBIs on monocots and dicots as compared with their untreated counterparts. Note the lesser degree of inhibition observed for some CBIs against monocots (discussed in text). CBI treatment manifests itself in a dwarfed phenotype often with ectopic lignification (see insert above CBI treated dicot). (B) CBI treated plant cells become radially swollen and irregularly shaped, which is visualized microscopically using a PIP2::GFP (plasma membrane reporter) and laser scanning confocal microscopy (Xia *et al.*, 2014). Scale bar = 10 μ m. (C) The use of radioisotope tracer studies to track the amount of incorporation into cellulose is common in defining a CBI. Here a CBI treated and untreated batch of seedlings are examined by 14 C glucose uptake and incorporation into cellulose. Inset is a batch of dark grown 7 d old etiolated seedlings that were grown in liquid culture prior to spiking with 14 C-glucose and a CBI or no CBI (scale is indicated by the 1 cm squares on the dish). (D) CBI elicits behavioral change in populations of CESA particles at the plasma membrane, cortex, or Golgi. Here, laser scanning or spinning disc confocal microscopy is used to image plants expressing RFP::TUA (red) and YFP::CESA6 (yellow) in CBI treated and untreated plants. As is shown in the left versus right panel comparisons, the treated results in the clearance of the CESA from the plasma membrane focal plane as apparent in the merged image (scale bar = 10 μ m). In the not treated panel, the CESA particles at the PM create linear tracks (white carats). By contrast, in the treated panel where PM bound CESA is absent, the intracellular compartments are localized as either Golgi (pink carats) or SMACs (yellow carats).

indaziflam, and acetobixan (Brabham *et al.*, 2014; Xia *et al.*, 2014; Worden *et al.*, 2015). Below we elaborate on the classification system and its potential use in understanding newly identified CBIs and complexity of cellulose biosynthesis.

Recently characterized CBIs

Indaziflam

One of the interesting CBIs to be added to both the commercial and research space was indaziflam. Indaziflam, a member of the **alkylazine family** is active at μ M concentrations and has a long soil residual making it an outstanding pre-emergent herbicide. The alkylazine scaffold has shown to be an excellent lead compound for CBI discovery and optimization. This **group includes indaziflam, triaziflam, and AE F150944** (Grossman *et al.*, 2001; Kiedaish *et al.*, 2003; Brabham *et al.*, 2014). **However, relating structure to MOA within this group has been difficult as the inhibitory mechanism of triaziflam, AE F150944, and indaziflam do not appear to match** (Grossman *et al.*, 2001; Kiedaish *et al.*, 2003; Guterriez *et al.*, 2009; Brabham *et al.*, 2014). Interestingly, indaziflam treatment induced an increase in the number of fluorescently labeled CESAs particles at the PM (~30%), but these particles exhibited reduced velocity (by approximately 66%) in

comparison to the untreated control CESAs (Brabham *et al.*, 2014). Colocalization rates between microtubules and CESAs were nearly abolished upon indaziflam treatment (53% compared with 70% in untreated), but this could be partially attributed to the increase in CESA particles at the PM. Since this phenotype was quite similar to CESA behavior observed in *csi1-3* mutants (Gu *et al.*, 2010; Lei *et al.*, 2012), the authors asked whether indaziflam treatment phenotypes would be visible in the *csi1-3* mutant background. No discernable differences were detected between the behavior of CESAs in wild type or *csi1-3*. It was concluded that the inhibitory mechanism of indaziflam does not require a functional CSI1, however, this does not exclude the potential requirement of CSI3 on CESA PM velocity. CSI3 is a homolog to CSI1 that also associates with the primary CSC and like CSI1 is required for normal velocity of the CSC as it moves along the microtubule tracks (Lei *et al.*, 2013). The exact role of CSI3 is unknown and while it is not redundant with CSI1 it is dependent on it for its proper function (Lei *et al.*, 2013). It would be of interest to see the effects of indaziflam on the *csi1 csi3* double mutant.

In contrast to indaziflam, AE F150944 (Kiedaish *et al.*, 2003) appears to cause different subcellular symptomologies than indaziflam. AE F150944 treatment induced clearance of CESA particles from the PM focal plane with no noticeable

influence on microtubule association (Gutierrez *et al.*, 2009). Furthermore, freeze fracture electron microscopy images showed AE F150944 treatment caused the relatively few CSC observed at the PM to become fractured (Kiedaish *et al.*, 2003), possibly a prelude to endocytosis and clearance from the PM. For triaziflam, no confocal or freeze fracture TEM images exist (Grossman *et al.*, 2001).

CESTRIN

Another newcomer to the CBI family is CESTRIN (CESA Trafficking Inhibitor) (1-[2,6-dinitro-4-(trifluoromethyl)phenyl]-2-[6-methyl-4-(trifluoromethyl)pyridin-2-yl]hydrazine). CESTRIN was identified (Drakakaki *et al.*, 2011; Worden *et al.*, 2015) and found to be an efficacious CESA exocytosis inhibitor (Worden *et al.*, 2015) that altered CSC trafficking in etiolated Arabidopsis hypocotyl cells from a screen of known pollen germination or endocytosis inhibitors (Drakakaki *et al.*, 2011). Worden and authors (2015) showed that CESTRIN is not a broad trafficking disruptor, but is specific to the proteins associated with CSC trafficking. CESTRIN largely reduced the number of CSCs in the PM and those that were present displayed reduced movement. Accompanying these phenotypes, CESTRIN treatment preferentially increased the abundance of CESAs in Syntaxin of Plants 61 (SYP61) intercellular labeled compartments. SYP61 is involved in the trafficking of vesicles from the trans-Golgi network to the PM (Sanderfoot *et al.*, 2001; Drakakaki *et al.*, 2012). SYP61 has been found to co-localize with intercellular compartmentalized CSC (Crowell *et al.*, 2009; Gutierrez *et al.*, 2009) and through proteomic analysis of proteins found in SYP61 labeled vesicles (Drakakaki *et al.*, 2012). As described above, CESTRIN also influenced the PM population of CSC particles. After CESTRIN treatment, most of the CSC particles were cleared from the PM but some CSCs remained visible (Worden *et al.*, 2015). However, the appearance of some CESAs at the PM can possibly be attributed to the relatively low rate used in this study (~3X) in comparison to other CBI studies (> 50X; X = rate reducing growth by 50%) (Crowell *et al.*, 2009; Gutierrez *et al.*, 2009). It is interesting that the MOA caused a slowdown in the remnant CSC particle movement at the PM. This may indicate a requirement for a specific CSC delivery rate or density to achieve normal movement. Alternatively, it may be that isoxaben and other CBIs that induce clearance (discussed in Group 1 below) all cause a slowdown of CSCs in the PM prior to complete clearance, which will be interesting to test experimentally.

Acetobixan

Acetobixan was discovered using subtractive metabolic fingerprinting from bacterial secretions (Xia *et al.*, 2014). Specifically, the lead compound was isolated from a library of complex bacterial secretions and refined to one that induced synergistic reduction in root growth in the *AtcesA6^{pre1-1}* mutant compared with wild type seedlings (with and without treatment). Similar to several other CBI compounds described below as clearance compounds (Group 1), acetobixan caused

clearance of YFP:CESA6 particles from the PM (Fig. 1D). Interestingly, mutants conferring resistance to quinoxiphen (Harris *et al.*, 2012) or isoxaben (Scheible *et al.*, 2001) were not cross resistant to acetobixan. These data infer that these CBIs may differentially disrupt the cellulose biosynthesis process.

Below, we explore the CBI classification system focusing on live cell confocal microscopy imaging of CESAs upon CBI treatment (Fig. 2A, B). We explore the potential to use confocal microscopy to study newly identified CBIs MOA, assess similarity between CBIs MOAs, and provide insights into the complexity of cellulose biosynthesis.

CBI classification system

Group 1: clearance of CSC from the PM focal plane

Compounds in Group 1 are based on the phenotype (cellular MOA) of fluorescently labeled CESA-containing CSCs being depleted from the PM focal plane and concomitantly accumulate in cytosolic vesicles. It is likely that all members of this group in fact elicit this phenotype but may do so by different mechanisms. Furthermore, fluorescently labeled CESAs are visually being produced in the Golgi (donut-shaped fluorescence in images), but in one way or another fail to reach and be inserted into the PM. This was demonstrated clearly for the well studied CBI isoxaben (Paredes *et al.*, 2006; Gutierrez *et al.*, 2009). Compounds in this group include isoxaben (Gutierrez *et al.*, 2009), quinoxiphen (Harris *et al.*, 2012), AE F150944 (Gutierrez *et al.*, 2009), CGA 325'615 (Crowell *et al.*, 2009), thaxtomin A (Bischoff *et al.*, 2010) and two new compounds CESTRIN (Worden *et al.*, 2015) and acetobixan (Xia *et al.*, 2014).

The molecular target of some members of Group 1 has been directly associated with CESAs (Fig. 3). Here, forward genetic screens are conducted using ethyl methyl sulfonate (EMS) mutagenized populations of Arabidopsis seed to look for resistance to CBIs among hundreds of thousands of individuals. From these screens, researchers have mapped resistance to multiple point mutations in *AtCESA1*, three or six that confer resistance to isoxaben (Scheible *et al.*, 2001; Desprez *et al.*, 2002; Sethaphong *et al.*, 2013) or quinoxiphen (Harris *et al.*, 2012; Sethaphong *et al.*, 2013) (Fig. 2). Resistance to the triazole carboxamides, triazofenamide and its more potent derivative flupoxam, has also been mapped to point mutation in *AtCESA1* and *AtCESA3* (Austin *et al.*, 2011; Shim, 2014). Although, flupoxam and triazofenamide meet the criteria to be classified as CBIs (Heim *et al.*, 1998; Kudo *et al.*, 1999; García-Angulo *et al.*, 2012), their effect on fluorescently labeled CESAs is unknown. It will be interesting to examine their MOA by confocal microscopy in the future to determine their influence on CESA.

An alternative scenario where the PM can become devoid of CESAs is a result of severe alteration in the trafficking of CESA-containing vesicles between the trans-Golgi network and the PM (Drakakaki *et al.*, 2011; Worden *et al.*, 2015). Delivery of CESA-containing vesicles to the PM is a highly coordinated

A.

Group 1: Clearance of CSC from the Plasma membrane focal plane	
Isoxaben	Gutierrez et al., 2009
Quinoxiphen	Harris et al., 2012
AE F150944	Gutierrez et al., 2009
CGA 325'615	Crowell et al., 2009
Thaxtomin-A	Bischoff et al., 2010
Flupoxam	Kudo et al., 1999
Triazofenamide	Heim et al., 1998
CESTRIN	Drakakaki et al., 2011
Worden et al., 2015	
Acetobixan	Xia et al., 2014
Group 2: Arrested CESA movement in the Plasma membrane	
DCB	Sabba et al., 1999
Indaziflam	Brabham et al., 2014
Group 3: Modifying CESA trajectory at the plasma membrane	
Morlin	DeBolt et al., 2007a
Cobtorin	Yoneda et al., 2007

B. Classification system of CBIs

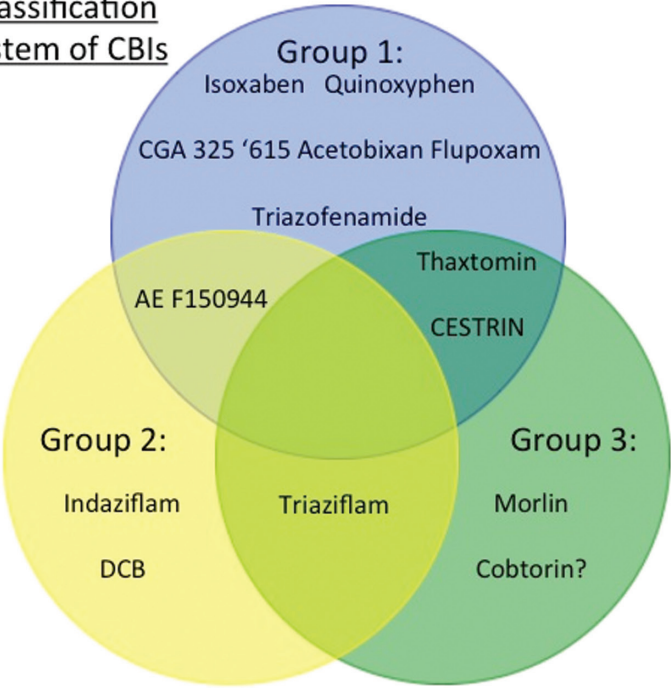


Fig. 2. List of CBIs and associated publications. (A) A table organizing the three groups of CBIs and their key papers describing their functions. (B) A Venn diagram depicting the three groups of CBIs and the complex nature of the classification system. The overlapping regions represent how a CBI can show a range of MOA that can pose a challenge to their classification. Note that the question mark next to cobtorin indicates that while Yoneda *et al.* (2007) found evidence for irregular cellulose deposition trajectories, live cell imaging of a CESA-reporter is needed to validate this classification.

process and is facilitated by microtubules and cargo transport proteins. Several advances have recently been made in this research area (Crowell *et al.*, 2009; Gutierrez *et al.*, 2009; Gu *et al.*, 2010; Drakakaki *et al.*, 2012; Bashline *et al.*, 2013; Lei *et al.*, 2013). Cortical microtubules, CSI1, CSI3, an adaptin protein μ 2, and the SYP61 have all been shown to partially coincide with CESA-containing vesicles indicating their importance in CESA trafficking and therefore could be viable CBI targets.

Thaxtomin A is a CBI identified from necrotrophic *Streptomyces spp.* Resistance to thaxtomin A was previously mapped to a protein of an unknown function (Scheible *et al.*, 2003). This protein was recently identified and characterized as the mitochondrial inner membrane protein import motor subunit called PAM16 (Huang and Fu, 2013). The authors concluded the loss of AtPAM16 limited the over-accumulation of reactive oxygen species required for cell death and

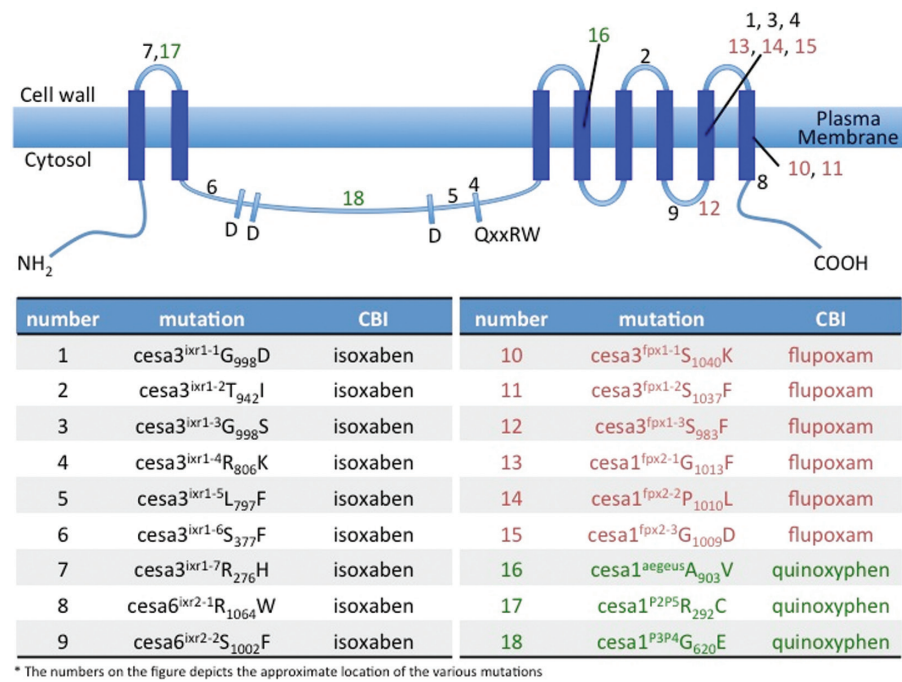


Fig. 3. Mutations in CESA confer resistance to CBIs. (A) Plant CESA diagram depicting the predicted eight transmembrane helices and the cytosolic catalytic region. (Note: diagram not to scale; for scale model of CESA of the cytoplasmic region refer to: Sethaphong et al., 2013). The diagram is a visual representation of the locations of the multiple published point mutations that have been demonstrated to confer resistance to CBIs. The point mutations listed confer resistance to CBIs within the primary cell wall CESAs (CESA1,3,6) (Heim et al., 1989; Scheible et al., 2001; Desprez et al., 2002; Harris et al., 2012; Sethaphong et al., 2013; Shim, 2014). The number on the diagram corresponds to the tabular listing of mutations below the schematic, to help the reader identify the exact location of the point mutation.

thus provided resistance to *Streptomyces spp.* but not necessarily thaxtomin A (Huang and Fu, 2013). Therefore, it was likely the CBI tolerance was a secondary effect. Questions about the CBI activity of thaxtomin A and the internalization of PM bound CESAs remain unanswered. One possible theory is thaxtomin A activates the early endocytosis CESA-related pathway that has been associated with SYP61 and other accessory proteins (Zhu et al., 2002; Robert et al., 2008; Crowell et al., 2009; Gutierrez et al., 2009; Drakakaki et al., 2012; Luo et al., 2015). To corroborate this, supplementing treated plant with auxin-like compounds has been shown to ameliorate the toxicity of thaxtomin A (King and Calhoun, 2009; Tegg et al., 2013). Interestingly, auxin transport proteins (PIN2) have also been shown to be endocytosed by the SYP61-trafficking complex (Robert et al., 2008). Further research is needed to see if SYP61-sensitive proteins, for example BRI1 and PIN2, are sensitive to thaxtomin A induced internalization.

Group 2: increased CESA in PM and slowed or arrested movement

The second CESA classification group contains DCB (2,6-dichlorobenzonitrile) and indaziflam (Fig. 2B). The CESA phenotype induced by these CBIs is interesting in that more CESA particles accumulate at the PM (Herth, 1987; DeBolt et al., 2007b). Accompanying this increase in CESA abundance is an almost complete reduction of CSC velocity (DeBolt et al., 2007b), and while indaziflam (Brabham et al., 2014) too accumulated more PM bound CESA particles it

elicits some interesting differences. DCB caused increased accumulation at specific foci at the PM focal plane resulting in brighter and brighter fluorescent ‘dots’ over a time series of 2h. By contrast, indaziflam induced a more even distribution of particles across the PM. Another variation from DCB was that indaziflam treatment resulted in a reduction rather than cessation of CESA particle movement at the PM. Obtaining resistant mutants using a mutagenesis approach has been unsuccessful for both. To date, we have screened at least 20-times the number of mutagenized seed for indaziflam resistance than used to isolate several quinoxiphen resistant mutants (Harris et al., 2012; Brabham and DeBolt, unpublished). Similarly, no DCB resistant mutants have been identified despite similar efforts. A modestly tolerant (2–4X) DCB mutant DH75 was reported by Heim and coworkers (1989), which would be interesting to examine further.

Group 3: modifying CESA trajectory to and at the PM

No additional CBIs that fall under the designation of compounds that alters the trajectory of CESA particles to and at the PM have arisen in the past few years. The main CBI in this group is morlin (7-ethoxy-4-methyl chromen-2-one) (DeBolt et al., 2007a). Notably, morlin has the potential to elicit its primary influence on microtubules, which could in turn influence trajectories of CSCs at the PM. While indirect evidence also exists for another CBI named cobtorin (4-[(2-chlorophenyl)-methoxy]-1-ntirobenzene) (Yoneda et al., 2007, 2010), it has not been used in combination with YFP::CESA6 or other live cell CSC reporter. Cobtorin alters

the methylation ratio and the distribution of pectin in the cell wall and was hypothesized to act by interfering with cellulose pectin associations. Resistance to cobtorin was also conferred by overexpressing a pectin biosynthetic gene (Yoneda *et al.*, 2007, 2010). We tentatively place cobtorin as a Group 3 CBI based on existing cellulose and microtubule imaging data (Yoneda *et al.*, 2007) but further work is needed to examine it in Arabidopsis cells expressing YFP:CESA6 and compare its MOA with morlin.

Difference in sensitivity to CBIs seen in monocotyledons versus dicotyledons

There has been a general trend for CBIs to inhibit dicot root elongation at lower rates as compared with monocots (Corio-Costet *et al.*, 1991; Sabba and Vaughn, 1999). This peculiarity could be due to a number of reasons such as seed size, metabolism, sequestration, herbicide uptake and translocation, or differences in the genetic composition. While seed size and metabolic differences are valid rationales, studies in plant tissue cultures have shown that tolerance to isoxaben in soybean nor wheat callus could not be explained simply by its metabolism or metabolic fate (Corio-Costet *et al.*, 1991).

Alternatively, could the composition of the cell wall also influence CBI tolerance? For example, the primary cell wall composition varies between certain plants with dicots and liliaceous monocots having type I cell walls while type II cell walls are found only in the Poales (grasses) and related commelinid monocots (Carpita and Gibeau, 1993; Carpita and McCann, 2008). When maize tissue and barley cultures (calli) are habituated in DCB and their cell wall analyzed, it was found that it was reduced in cellulose content, but increased in mixed linkage glucans or glucuronoarabinoxylan (GAX) and arabinoxylans, and it was hypothesized that the increase in cell wall phenolics could be a compensation mechanism for the 'cellulose impoverished cell wall' (Shedletzky *et al.*, 1992; Mérida *et al.*, 2010). If the cellulose biosynthetic carbon sink is halted, where does the metabolic pool destined for cellulose production go? ¹⁴C glucose uptake studies suggest that it can be diverted to pectin and hemicelluloses (García-Angulo *et al.*, 2012). This could be signifying a compensation mechanism in which the excess glucose is being utilized for hemicellulose (xyloglucan, heteroxylan) production in grasses and pectin production in dicots. With the notable differences in cell wall composition in the grasses, this diversion to alternative cell wall polysaccharides caused by the CBIs could differentially influence the response. Understanding this divergence will be interesting for the cellulose biosynthesis research community but also the broader weed science community.

CBIs and the across kingdom view of cellulose synthesis

The terminal complex extruding cellulose has significantly evolved overtime from a single linear array in the prokaryote *Gluconacetobacter xylinus* (Ross *et al.*, 1991) to the solitary, hexagonal, rosette-shaped complex in land plants (Tsekos,

1999). We postulate that this divergence possibly explains the selectivity of CBIs towards plants, except for the non-selective nature of DCB on cellulose producing eukaryote (Mizuta and Brown, 1992; Orologas *et al.*, 2005; DeBolt *et al.*, 2007b). Another class of CBIs, the carboxylic acid amides (CAAs), has been commercialized to control cellulose producing oomycetes, for example *Phytophthora infestans* (Blum *et al.*, 2010). There are no freeze fracture electron microscopy images of the terminal complex in oomycetes, but the C-terminus of PiCESAs has a similar predicted protein topology to plant CESAs (Somerville, 2006; Grenville-Briggs *et al.*, 2008). Point mutations conferring resistance to CAAs have been mapped to the C-terminus of CESA3 in several oomycetes (Blum *et al.*, 2012).

In cellulose producing prokaryotes, BcsA (bacterial CESA) is 'activated' with the binding of the allosteric agonist cyclic-di-GMP (Amikam and Galperin, 2006; Morgan *et al.*, 2014). In the absence of cyclic-di-GMP, the catalytic pocket is blocked by interface helices 3 (IF3) (between transmembrane helices 6 and 7 in BcsA) referred to as the gating loop, and is sterically hindered by the cytosolic C-terminus of BcsA. This inhibition is removed by a conformational change in the C-terminus upon cyclic-di-GMP binding (Morgan *et al.*, 2014). However in eukaryotic CESAs, the cyclic-di-GMP binding site has been lost along with the majority of the cytosolic C-terminus, but the gating loop core sequence has remained fairly conserved (the amino acid residues FxVTxK in the IF between transmembrane helices 5 and 6 in Arabidopsis) (Slabaugh *et al.*, 2014). The presence of such a gating loop has yet to be established in eukaryotic CESAs and may not exist. The clustering of CAA- and Group 1 CBI-resistant point mutations in the putative pore-forming transmembrane domains of CESA orthologs (Blum *et al.*, 2012; Sethaphong *et al.*, 2013) begs the question of whether CESAs are under allosteric control (in the absence of CBIs) and what is the ligand?

CBIs and their subsequent resistant point mutations have proved useful in examining question in the absence of a crystallized plant CESA. For instance, the putative gating loop region (Slabaugh *et al.*, 2014) has been shown to be required for AtCESA1 function. Here, an amino acid substitution from the conserved Phe to a Leu at position 954 in the gating loop resulted in dysfunctional CSCs (Slabaugh *et al.*, 2014). This was further supported with live cell imaging of the mutated variant showing fluorescently labeled AtCESA1^{F954L} was not found to accumulate at the PM focal plane. It is important to note that null mutations in AtCESA1 are lethal and therefore transformations and experiments had to be performed at restrictive temperatures in the temperature sensitive *rws1* mutant background. Furthermore, in AtCESA3 a Thr to Ile substitution at position 942 in the conserved region of the gating loop confers a high level resistance to isoxaben. While a T942I in AtCESA3 does not disrupt protein function, it does have a significant effect on cellulose crystallinity (Harris *et al.*, 2012; Griffiths *et al.*, 2015).

The putative gated loop region appears important for CESA function. Could this region be important for the inhibitory mechanism of Group 1 CBIs? Analysis of computational

data suggests that this region exists in a binary state as either 'up' or 'down' (Slabaugh *et al.*, 2014). The two amino acid substitutions examined influence the preferred position, with the F954L favoring the conformational 'down' state, while T942I favored an 'up' state (Slabaugh *et al.*, 2014). Based on this information, one possibility is that Group 1 CBIs act as steric inhibitors by preventing conformational change of CESAs from a 'off' to an 'on' state. This could explain why treatment with Group 1 CBIs results in a PM devoid of CSC as the complex is in an 'off' state. On the other hand, if this region is constitutively down or 'on' then a given CBI i.e. isoxaben may not bind to its cognate target. However, such mechanisms remain purely speculative and perhaps may be best aided by molecular dynamic simulations of the plant CESAs (Sethaphong *et al.*, 2013) since no crystal structures are available.

Conclusion

Combining genetics with CBIs will continue to assist in elucidating the basic mechanisms of cellulose and cell wall biogenesis, and continued development of new and current CBIs is expected to be driven by their utility in cellulose biosynthesis research but also as weed control agents. The capacity for new inhibitory mechanisms of action in the broad CBI grouping is particularly of interest due to the lack on new herbicidal MOAs developed in the past decades. Additionally, breakthroughs in advanced cellular imaging techniques will also facilitate the use of CBIs as research tools to disrupt cellulose biosynthesis in a targeted way. Beyond cellulose, using chemical genetics to dissect other cell wall processes is anticipated. We highlight that screening natural compounds for future CBIs (Bischoff *et al.*, 2010; Xia *et al.*, 2014) may also be valuable to identify new MOAs.

References

- Amikam D, Galperin MY. 2006. PilZ domain is part of the bacterial c-di-GMP binding protein. *Bioinformatics* **22**, 3–6.
- Austin RS, Vidaurre D, Stamatiou G, *et al.* 2011. Next-generation mapping of Arabidopsis genes. *The Plant Journal* **67**, 715–725.
- Bashline L, Li S, Anderson CT, Lei L, Gu Y. 2013. The endocytosis of cellulose synthase in Arabidopsis is dependent on μ 2, a clathrin-mediated endocytosis adaptin. *Plant Physiology* **163**, 150–160.
- Baskin TI. 2005. Anisotropic expansion of the plant cell wall. *Annual review of cell and developmental biology* **21**, 203–222.
- Bischoff V, Nita S, Neumetzler L, Schindelasch D, Urbain A, Eshed R, Persson S, Delmer D, Scheible W-R. 2010. TRICHOME BIREFRINGENCE and its homolog AT5G01360 encode plant-specific DUF231 proteins required for cellulose biosynthesis in Arabidopsis. *Plant Physiology* **153**, 590–602.
- Blum M, Boehler M, Randall E, *et al.* 2010. Mandipropamid targets the cellulose synthase-like PICESA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. *Molecular Plant Pathology* **11**, 227–243.
- Blum M, Gamper H A, Waldner M, Sierotzki H, Gisi U. 2012. The cellulose synthase 3 (CesA3) gene of oomycetes: Structure, phylogeny and influence on sensitivity to carboxylic acid amide (CAA) fungicides. *Fungal Biology* **116**, 529–542.
- Brabham C, DeBolt S. 2012. Chemical genetics to examine cellulose biosynthesis. *Frontiers in Plant Science* **3**.
- Brabham C, Lei L, Gu Y, Stork J, Barrett M, DeBolt S. 2014. Indaziflam Herbicidal Action: A Potent Cellulose Biosynthesis Inhibitor. *Plant Physiology* **166**, 1177–1185.
- Bringmann M, Li E, Sampathkumar A, Kocabek T, Hauser M-T, Persson S. 2012. POM-POM2/CELLULOSE SYNTHASE INTERACTING1 Is Essential for the Functional Association of Cellulose Synthase and Microtubules in Arabidopsis. *The Plant Cell* **24**, 163–177.
- Carpita NC, Gibeaut DM. 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 Journal* **3**, 1–30.
- Carpita NC, McCann MC. 2008. Maize and sorghum: genetic resources for bioenergy grasses. *Trends in plant science* **13**, 415–420.
- Carpita NC. 2011. Update on mechanisms of plant cell wall biosynthesis: how plants make cellulose and other (1–4)- β -D-glycans. *Plant Physiology* **155**, 171–184.
- Chen S, Ehrhardt DW, Somerville CR. 2010. Mutations of cellulose synthase (CESA1) phosphorylation sites modulate anisotropic cell expansion and bidirectional mobility of cellulose synthase. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 17188–17193.
- Corio-Costet M-F, Agnese MD, Scalla R. 1991. Effects of Isoxaben on Sensitive and Tolerant Plant Cell Cultures I. Metabolic fate of isoxaben. *Pesticide Biochemistry and Physiology* **40**, 246–254.
- Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof Y-D, Schumacher K, Gonneau M, Höfte H, Vernhettes S. 2009. Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in Arabidopsis. *The Plant cell* **21**, 1141–1154.
- DeBolt S, Gutierrez R, Ehrhardt DW, Melo CV, Ross L, Cutler SR, Somerville C, Bonetta D. 2007a. Morlin, an inhibitor of cortical microtubule dynamics and cellulose synthase movement. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 5854–5859.
- DeBolt S, Gutierrez R, Ehrhardt DW, Somerville C. 2007b. Nonmotile cellulose synthase subunits repeatedly accumulate within localized regions at the plasma membrane in Arabidopsis hypocotyl cells following 2,6-dichlorobenzonitrile treatment. *Plant Physiology* **145**, 334–338.
- Desprez T, Vernhettes S, Fagard M, Refrégier G, Desnos T, Aletti E, Py N, Pelletier S, Höfte H. 2002. Resistance against herbicide isoxaben and cellulose deficiency caused by distinct mutations in same cellulose synthase isoform CESA6. *Plant Physiology* **128**, 482–490.
- Desprez T, Juraniec M, Crowell EF, Jouy H, Pochylova Z, Parcy F, Höfte H, Gonneau M, Vernhettes S. 2007. Organization of cellulose synthase complexes involved in primary cell wall synthesis in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 15572–7.
- Drakakaki G, Robert S, Szatmari a.-M, *et al.* 2011. Clusters of bioactive compounds target dynamic endomembrane networks in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 17850–17855.
- Drakakaki G, van de Ven W, Pan S, *et al.* 2012. Isolation and proteomic analysis of the SYP61 compartment reveal its role in exocytic trafficking in Arabidopsis. *Cell Research* **22**, 413–424.
- Endler A, Kesten C, Schneider R, Zhang Y, Ivakov A, Froehlich, A, Funke N, Persson, S. 2015. A Mechanism for Sustained Cellulose Synthesis during Salt Stress. *Cell* **162**, 1353–1364.
- Fernandes A N, Thomas LH, Altaner CM, Callow P, Forsyth VT, Apperley DC, Kennedy CJ, Jarvis MC. 2011. Nanostructure of cellulose microfibrils in spruce wood. *Proceedings of the National Academy of Sciences of the United States of America* **108**.
- García-Angulo P, Alonso-Simón A, Encina A, Álvarez JM, Acebes JL. 2012. Cellulose biosynthesis inhibitors: Comparative effect on bean cell cultures. *International Journal of Molecular Sciences* **13**, 3685–3702.
- Gonneau M, Desprez T, Guillot A, Vernhettes S, Höfte H. 2014. Catalytic Subunit Stoichiometry within the Cellulose Synthase Complex. *Plant Physiology* **166**, 1709–1712.
- Grenville-Briggs LJ, Anderson VL, Fugelstad J, *et al.* 2008. Cellulose synthesis in *Phytophthora infestans* is required for normal appressorium formation and successful infection of potato. *The Plant Cell* **20**, 720–738.

- Griffiths J, Šola K, Kushwaha R, et al.** 2015. Unidirectional movement of cellulose synthase complexes in Arabidopsis seed coat epidermal cells deposit cellulose involved in mucilage extrusion, adherence and ray formation. *Plant Physiology*, doi: <http://dx.doi.org/10.1104/pp.15.00478>
- Grossmann K, Tresch S, Plath P.** 2001. Triaziflam and diaminotriazine derivatives affect enantioselectively multiple herbicide target sites. *Zeitschrift für Naturforschung C* **56**, 559–569.
- Gu Y, Kaplinsky N, Bringmann M, Cobb A, Carroll A, Sampathkumar A, Baskin TI, Persson S, Somerville CR.** 2010. Identification of a cellulose synthase-associated protein required for cellulose biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 12866–12871.
- Gutierrez R, Lindeboom JJ, Paredez AR, Emons AMC, Ehrhardt DW.** 2009. Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nature Cell Biology* **11**, 797–806.
- Haigler CH, Brown Jr R.** 1986. Transport of Rosettes from the Golgi Apparatus to the Plasma Membrane in Isolated Mesophyll Cells of *Zinnia elegans* During Differentiation to Tracheary Elements in Suspension Culture. *Protoplasma* **134**, 111–120.
- Harris D, Stork J, Debolt S.** 2009. Genetic modification in cellulose-synthase reduces crystallinity and improves biochemical conversion to fermentable sugar. *GCB Bioenergy* **1**, 51–61.
- Harris DM, Corbin K, Wang T, et al.** 2012. Cellulose microfibril crystallinity is reduced by mutating C-terminal transmembrane region residues CESA1A903V and CESA3T942I of cellulose synthase. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 4098–103.
- Heim DR, Roberts JL, Pike PD, Larrinua IM.** 1989. Mutation of a Locus of Arabidopsis thaliana Confers Resistance to the Herbicide Isoxaben. *Plant physiology* **90**, 146–150.
- Heim DR, Larrinua IM, Murdoch MG, Roberts JL.** 1998. Triazofenamide is a cellulose biosynthesis inhibitor. *Pesticide Biochemistry and Physiology* **59**, 163–168.
- Herth W.** 1987. Effects of 2, 6-DCB on plasma membrane rosettes of wheat root cells. *Naturwissenschaften* **74**, 556–557.
- Hill JL, Hammudi MB, Tien M.** 2014. The Arabidopsis Cellulose Synthase Complex: A Proposed Hexamer of CESA Trimers in an Equimolar Stoichiometry. *The Plant Cell* **26**, 4834–4842.
- Huang YB, Fu Y.** 2013. Hydrolysis of cellulose to glucose by solid acid catalysts. *Green Chemistry* **15**, 1095–1111.
- Kiedaisch BM, Blanton RL, Haigler CH.** 2003. Characterization of a novel cellulose synthesis inhibitor. *Planta* **217**, 922–930.
- Kimura S, Laosinchai W, Itoh T, Cui X, Linder C, Brown R.** 1999. Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *vigna angularis*. *The Plant Cell* **11**, 2075–2086.
- King RR, Calhoun LA.** 2009. The thaxtomin phytotoxins: sources, synthesis, biosynthesis, biotransformation and biological activity. *Phytochemistry* **70**, 833–841.
- Kudo NK, Furuta SF, Taniguchi MT, Endo TE, Sato KS.** 1999. Synthesis and Herbicidal Activity of 1, 5-Diarylpyrazole Derivatives. *Chemical and Pharmaceutical Bulletin* **47**, 857–868.
- Lane DR, Wiedemeier A, Peng L, et al.** 2001. Temperature-sensitive alleles of RSW2 link the KORRIGAN endo-1,4-beta-glucanase to cellulose synthesis and cytokinesis in Arabidopsis. *Plant Physiology* **126**, 278–288.
- Lei L, Li S, Gu Y.** 2012. Cellulose Synthase Complexes: Composition and Regulation. *Frontiers in Plant Science* **3**, 1–6.
- Lei L, Li S, Du J, Bashline L, Gu Y.** 2013. CELLULOSE SYNTHASE INTERACTIVE3 Regulates Cellulose Biosynthesis in Both a Microtubule-Dependent and Microtubule-Independent Manner in Arabidopsis. *The Plant Cell* **25**, 4912–23.
- Li S, Bashline L, Lei L, Gu Y.** 2014. Cellulose synthesis and its regulation. *The Arabidopsis book / American Society of Plant Biologists* **12**, e0169.
- Luo Y, Scholl S, Doering A, et al.** 2015. V-ATPase activity in the TGN/EE is required for exocytosis and recycling in Arabidopsis. *Nature Plants* **1**.
- Mansoori N, Timmers J, Desprez T, Kamei CL a., Dees DCT, Vincken J-P, Visser RGF, Höfte H, Vernhettes S, Trindade LM.** 2014. KORRIGAN1 Interacts Specifically with Integral Components of the Cellulose Synthase Machinery. *PLoS ONE* **9**, e112387.
- Mélida H, García-Angulo P, Alonso-Simón A, Álvarez JM, Acebes JL, Encina A.** 2010. The phenolic profile of maize primary cell wall changes in cellulose-deficient cell cultures. *Phytochemistry* **71**, 1684–1689.
- Mizuta S, Brown Jr RM.** 1992. High resolution analysis of the formation of cellulose synthesizing complexes in *Vaucheria hamata*. *Protoplasma* **166**, 187–199.
- Morgan JL, McNamara JT, Zimmer J.** 2014. Mechanism of activation of bacterial cellulose synthase by cyclic di-GMP. *Nature Structural & Molecular Biology* **21**, 489–496.
- Orologas N, Delivopoulos SG, Dimopoulou A, Tsekos I.** 2005. Effects of 2, 6-dichlorobenzonitrile on plasma membrane cellulose synthesizing complexes and cellulose localization in cells of the red alga *Erythrocladia subintegra*. *Phycologia* **44**, 465–476.
- Paredez AR, Somerville CR, Ehrhardt DW.** 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312**, 1491–1495.
- Persson S, Paredez A, Carroll A, Palsdottir H, Doblin M, Poindexter P, Khitrov N, Auer M, Somerville CR.** 2007. Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 15566–71.
- Robert S, Chary SN, Drakakaki G, Li S, Yang Z, Raikhel NV, Hicks GR.** 2008. Endosidin1 defines a compartment involved in endocytosis of the brassinosteroid receptor BRI1 and the auxin transporters PIN2 and AUX1. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 8464–8469.
- Ross P, Mayer R, Benziman M.** 1991. Cellulose biosynthesis and function in bacteria. *Microbiological reviews* **55**, 35–58.
- Roudier F, Fernandez AG, Fujita M, et al.** 2005. COBRA, an Arabidopsis extracellular glycosyl-phosphatidyl inositol-anchored protein, specifically controls highly anisotropic expansion through its involvement in cellulose microfibril orientation. *The Plant Cell* **17**, 1749–1763.
- Sabba RP, Vaughn KC.** 1999. Herbicides that inhibit cellulose biosynthesis. *Weed Science*, 757–763.
- Sanderfoot, AA, Kovaleva, V, Bassham, DC, Raikhel, NV.** 2001. Interactions between syntaxins identify at least five SNARE complexes within the Golgi/prevacuolar system of the Arabidopsis cell. *Molecular Biology of the Cell*, **12**, 3733–3743.
- Saxena IM, Brown RM.** 1997. Identification of cellulose synthase(s) in higher plants: sequence analysis of processive β -glycosyltransferases with the common motif "D, D, D35Q (R, Q) XRW". *Cellulose* **4**, 33–49.
- Scheible WR, Eshed R, Richmond T, Delmer D, Somerville C.** 2001. Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in Arabidopsis *lrx1* mutants. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 10079–10084.
- Scheible WR, Fry B, Kochevenko A, Schindelasch D, Zimmerli L, Somerville S, Loria R, Somerville CR.** 2003. An Arabidopsis mutant resistant to thaxtomin A, a cellulose synthesis inhibitor from *Streptomyces* species. *The Plant Cell* **15**, 1781–1794.
- Sethaphong L, Haigler CH, Kubicki JD, Zimmer J, Bonetta D, Debolt S, Yingling YG.** 2013. Tertiary model of a plant cellulose synthase. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 7512–7.
- Shedletzky E, Shmuel M, Trainin T, Kalman S, Delmer D.** 1992. Cell Wall Structure in Cells Adapted to Growth on the Cellulose-Synthesis Inhibitor 2,6-Dichlorobenzonitrile : A Comparison between Two Dicotyledonous Plants and a Gramineous Monocot. *Plant Physiology* **100**, 120–130.
- Shim I.** 2014. Forward Genetic Analysis of Cellulose Biosynthesis Inhibitor Resistance Wall Hydrolysis Sensitivity. University of Ontario Institute of Technology. <http://hdl.handle.net/10155/461>
- Slabaugh E, Sethaphong L, Xiao C, Amick J, Anderson CT, Haigler CH, Yingling YG.** 2014. Computational and genetic evidence that different structural conformations of a non-catalytic region affect the function of plant cellulose synthase. *Journal of Experimental Botany* **65**, 6645–6653.
- Somerville C, Bauer S, Brininstool G, et al.** 2004. Toward a Systems Approach to Understanding plant cell walls. *Science* **306**, 2206–2211.

- Somerville C.** 2006. Cellulose synthesis in higher plants. *Annual Review of Cell and Developmental Biology* **22**, 53–78.
- Sorek N, Sorek H, Kijac A, Szemenyei HJ, Bauer S, Hematy K, Wemmer DE, Somerville CR.** 2014. The Arabidopsis COBRA protein facilitates cellulose crystallization at the plasma membrane. *The Journal of Biological Chemistry* **289**, 34911–34920.
- Taylor NG, Howells RM, Huttly AK, Vickers K, Turner SR.** 2003. Interactions among three distinct CesA proteins essential for cellulose synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 1450–1455.
- Tegg RS, Shabala SN, Cuin TA, Davies NW, Wilson CR.** 2013. Enhanced resistance to the cellulose biosynthetic inhibitors, thaxtomin A and isoxaben in *Arabidopsis thaliana* mutants, also provides specific co-resistance to the auxin transport inhibitor, 1-NPA. *BMC Plant Biology* **13**, 76.
- Tsekos I.** 1999. The sites of cellulose synthesis in algae: diversity and evolution of cellulose-synthesizing enzyme complexes. *Journal of Phycology* **35**, 635–655.
- Vain T, Crowell EF, Timpano H, et al.** 2014. The cellulase KORRIGAN is part of the Cellulose Synthase Complex. *Plant Physiology* **165**, 1521–1532.
- Vogel J.** 2008. Unique aspects of the grass cell wall. *Current Opinion in Plant Biology* **11**, 301–307.
- Wang T, Hong M.** 2016. Solid-state NMR investigations of cellulose structure and interactions with matrix polysaccharides in plant primary cell walls. *Journal of Experimental Botany* **67**, 503–514.
- Worden N, Wilkop TE, Esteva Esteve V, et al.** 2015. CESTRIN, inhibits cellulose deposition and interferes with the trafficking of cellulose synthase complexes and their associated proteins KORRIGAN1 and POM2/CSI1. *Plant Physiology* **167**, 381–393.
- Xia Y, Petti C, Williams M A., DeBolt S.** 2014. Experimental approaches to study plant cell walls during plant-microbe interactions. *Frontiers in Plant Science* **5**, 1–7.
- Yoneda A, Higaki T, Kutsuna N, Kondo Y, Osada H, Hasezawa S, Matsui M.** 2007. Chemical genetic screening identifies a novel inhibitor of parallel alignment of cortical microtubules and cellulose microfibrils. *Plant and Cell Physiology* **48**, 1393–1403.
- Yoneda A, Ito T, Higaki T, Kutsuna N, Saito T, Ishimizu T, Osada H, Hasezawa S, Matsui M, Demura T.** 2010. Cobtorin target analysis reveals that pectin functions in the deposition of cellulose microfibrils in parallel with cortical microtubules. *Plant Journal* **64**, 657–667.
- Zhu J, Gong Z, Zhang C, Song C, Damsz B, Inan G, Koiwa H, Zhu J, Hasegawa PM, Bressan RA.** 2002. OSM1 / SYP61 : A Syntxin Protein in Arabidopsis Controls Absciscic Acid – Mediated and Non-Absciscic Acid – Mediated Responses to Abiotic Stress. *The Plant Cell* **14**, 3009–3028.