Introduction and Disclaimer

These mock examination questions span diverse disciplines and are designed for your practice in preparation for the International Research Olympiad (IRO) 2024. Endeavor to answer them to the best of your ability, utilizing this opportunity to enhance your skills and knowledge. For additional practice, it is advisable to engage in extensive reading of various papers; such efforts will contribute to a more comprehensive and nuanced understanding of the subject matter.

Try your best, and good luck! -International Research Olympiad 2024

Small but Mighty: An Update on Small Molecule Plant Cellulose Biosynthesis Inhibitors

Abstract

Cellulose is one of the most abundant biopolymers on Earth. It provides mechanical support to growing plant cells and important raw materials for paper, textiles and biofuel feedstocks. Cellulose biosynthesis inhibitors (CBIs) are invaluable tools for studying cellulose biosynthesis and can be important herbicides for controlling weed growth. Here, we review CBIs with particular focus on the most widely used CBIs and recently discovered CBIs. We discuss the effects of these CBIs on plant growth and development and plant cell biology and summarize what is known about the mode of action of these different CBIs.

Paper

Primary cell walls surround growing plant cells; in dicots, such as the model plant *Arabidopsis thaliana*, primary cell walls are mainly comprised of cellulose embedded in a hydrated matrix of pectins and hemicelluloses, plus some proteins. Secondary cell walls fortify specific cell types, such as vascular tissue, and are particularly enriched in cellulose, certain hemicelluloses and lignin. Cellulose is a homopolymer of many chains of β-(1,4)-linked glucose that laterally associate together into a cellulose microfibril, while pectins and hemicelluloses are heterogeneous classes of polymers comprised of a variety of monosaccharides and linkages.

Cellulose is made at the plasma membrane by the activity of cellulose synthases (CESAs). CESAs associate together to form a cellulose synthase complex (CSC) with 6-fold symmetry, presumably representing six CESA trimers per CSC. CESA assembly into CSCs positions the glucan chains synthesized by each CESA so that they can laterally associate to form a cellulose microfibril of 18 glucan chains from a single CSC. CESAs are encoded by multigene families, and multiple CESA isoforms are usually required for cellulose synthesis and plant viability, implying that CSC subunits are heterotrimers. In Arabidopsis, CESA1, CESA3 and one of the CESA6-like clade members (CESA2, CESA5, CESA6 and CESA9) are required for primary cell wall synthesis, while CESA4, CESA7 and CESA8 are required for secondary cell wall synthesis. CESAs are multi-pass transmembrane proteins, with seven transmembrane domains (TMs) and three interface helices (IFs); the intracellular N-terminus extends into the cytoplasm and may bridge interactions with CESA-associated proteins, while the C-terminus is extracellular. The catalytic residues reside in the cytosolic region between TM2 and TM3 and are conserved between plant and bacterial cellulose synthases. Plant CESAs contain several insertions, relative to bacterial cellulose synthases, including an N-terminal variable region (VR1), plus a plant-conserved region (PCR) and another highly variable region (VR2) in the cytosolic region between TM2 and TM3. These plant-specific regions are presumed to play important roles in CESA assembly into CSCs and interactions with CESA accessory proteins. When assembled into a trimer, TM4, TM6 and IF3 from one CESA interact with

TM7 from a neighboring CESA, and the N-termini and PCRs from different CESAs also interact, implicating these regions in CSC assembly.

Live cell imaging of fluorescent protein (FP)-tagged CESAs has revealed that CESAs are dynamically localized to the plasma membrane, the Golgi apparatus and to small CESA compartments (SmaCCs), which are either post-Golgi secretory compartments or endocytic compartments. CESAs are assembled into CSCs in the endoplasmic reticulum or Golgi apparatus and secreted to the plasma membrane via vesicle trafficking. Once in the plasma membrane, CSCs move in linear trajectories to synthesize cellulose; since glucose addition is coupled to glucan chain translocation across the membrane, CSCs must move to allow space for continued glucose residue addition. CSC movement is guided, in part, by microtubules at the cell cortex. Cellulose microfibril alignment is important for the biophysical properties of the cell wall and helps define the direction of cell expansion. Cellulose microfibril alignment and cellulose synthesis rates can be inferred by tracking the speed, direction and orientation of FP-CESA trajectories in the plasma membrane. Eventually, CESAs are inactivated and internalized via clathrin-mediated endocytosis; however, CESAs seem to cycle between the Golgi apparatus and the plasma membrane.

Cellulose biosynthesis inhibitors (CBIs) have been used to study cellulose synthesis in model plants, and some CBIs constitute important pre- and post-emergence herbicides. CBIs are excellent tools for studying plant cell wall synthesis since they can be used to disrupt cellulose synthesis in a reversible, concentration-dependent manner on a controllable timescale. CBIs are structurally diverse chemicals and they may be either natural or synthetic in origin, but they are all defined by their ability to cause a reduction in 14C-glucose incorporation into cellulose after a short-term (~2-hour) treatment. Therefore, CBI-treated plants phenocopy cellulose-deficient mutants, including decreased cellulose content, reduced cell elongation and cell swelling. These phenotypes may also include deposition of non-cellulosic cell wall-fortifying polymers, such as callose and/or lignin. Many CBIs also directly affect the localization, distribution or movement of FP-CESAs. The mode of CBI action can be defined by searching for resistance mutants or by studying CBI interactions with targeted proteins. We discuss CBIs here by comparing them to the most widely used and extensively studied CBI, isoxaben.

Isoxaben is a pre-emergence herbicide, originally used to control unwanted dicot growth and it is a potent CBI: 1 µM isoxaben treatment induced a 90% reduction in 14C-glucose incorporation into cellulose in Arabidopsis. Isoxaben-treated Arabidopsis seedlings displayed reduced growth, cell swelling and deposition of lignin and callose. Beyond Arabidopsis, isoxaben sensitivity is usually inferred based upon similar phenotypes, rather than via assays for 14C-glucose incorporation into cellulose. By this definition, isoxaben is active against many Archaeplastida (a eukaryotic supergroup containing plants and algae, defined by the acquisition of a plastid via endosymbiosis) with cell walls, with the exception of the crop grass species it was originally developed to protect (Figure 1). Outside of Archaeplastida isoxaben treatment decreased cellulose deposition and increased lysis susceptibility in *Pelvetia compressa*, implying that isoxaben may also be active on brown algae.

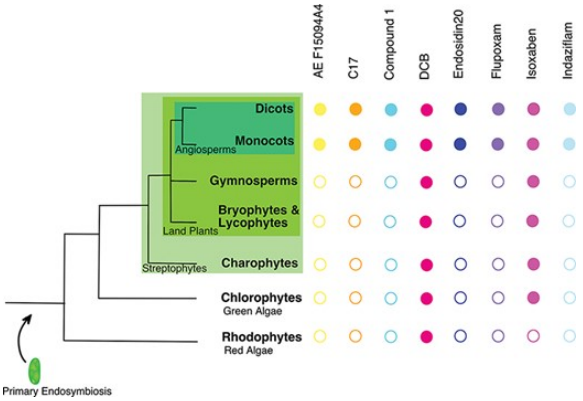


Figure 1: Breadth of CBI studies across Archaeplastid plants and algae. Phylogenetic representation of land plants and algae within the Archaeplastid eukaryotic supergroup (primary plastid-containing organisms), with key taxa indicated. A filled circle indicates that at least one peer-reviewed study has tested the indicated CBI on one species within the taxon. An open circle indicates no published studies have documented the effects of the CBI within that taxa.

Although isoxaben is active against a broad species range, grasses are relatively resistant to isoxaben. This tolerance did not seem to be the result of reduced isoxaben uptake or increased isoxaben metabolism. One hypothesis is that differences in isoxaben sensitivity might be due to differences in cell wall composition; many monocot grasses that display isoxaben resistance have cell walls that contain mixed-linkage glucan (MLG: β-(1,3;1,4)-glucan) in addition to cellulose (β-(1,4)-glucan). To test this hypothesis, Brabham et al. (2018) isolated knockdown mutants affecting *CSLF6*, one of the key genes involved in MLG synthesis in the model grass, *Brachypodium distachyon*. They found that *cslf6*-mutant Brachypodium plants with decreased cell wall MLG content were hypersensitive to isoxaben. These results imply that MLGs in grass cell walls may contribute to isoxaben resistance and suggest that differences in cell wall composition may account for other differences in CBI sensitivity across species.

In Arabidopsis, the subcellular location and dynamics of FP-CESAs were rapidly and dramatically affected by isoxaben, which implies that isoxaben directly targets CSCs. When Arabidopsis hypocotyl cells were treated with 100 nM isoxaben for only 20 minutes, FP-CESA6 was lost from the plasma membrane and internalized into SmaCCs. By contrast, in the bryophyte *Physcomitrium patens*, growth was unaffected by isoxaben treatment, even at micromolar concentrations, nor did isoxaben affect the density of CSCs in the plasma membrane but did reduce their speed.

While the effects of isoxaben on primary cell wall biosynthesis are well established, its effects on secondary cell wall biosynthesis are less clear. Treatment with 1 μM isoxaben induced a 75% reduction in 14C-glucose incorporation into cellulose during secondary cell wall biosynthesis in *Zinnia elegans* transdifferentiating culture cells. Isoxaben also caused loss of secondary cell wall FP-CESA7 signal in the plasma membrane of Arabidopsis root vascular tissue. By contrast, in Arabidopsis transdifferentiating cells, isoxaben caused internalization of primary cell wall FP-CESA6 into SmaCCs, but secondary cell wall FP-CESA7 remained in the plasma membrane. These differences may be due to differences in isoxaben concentrations, since Wightman and Turner (2008) and Kiedaisch et al. (2003) both used micromolar concentrations, while Watanabe et al. (2018) employed nanomolar concentrations, similar to the concentrations employed to study primary cell wall CESAs. It seems that secondary cell wall CESAs are at least an order of magnitude more resistant to isoxaben than primary cell wall CESAs, but the underlying reasons for these differences remain unclear.

Identification of isoxaben-resistant Arabidopsis mutants has provided strong evidence that isoxaben directly targets CESAs. Ten isoxaben-resistant mutants have been identified in Arabidopsis from multiple different screens (Figure 2). All 10 mutations map to *CESA3* or *CESA6*. Surprisingly, these mutations do not map to a single conserved position or region when CESA3 and CESA6 are aligned, although many are in the TMs, particularly TM4, TM6 and TM7. Interestingly, *CESA1* and *CESA3* appear to be most closely related and are speculated to have arisen from a recent and common duplication, so it is curious that isoxaben-resistant mutations have not been isolated in *CESA1*. If isoxaben associates with similar protein structures, then isoxaben resistance should be conferred by similar mutations in *CESA1* and *CESA3*. This does not appear to be the case, suggesting that a specific area of the three-dimensional structure of the CSC is being targeted by isoxaben, rather than a short sequence in an individual protein. In particular, the interaction between CESA3 and CESA6 may be this target. This hypothesis is consistent with recent structural information about CESA assembly into trimers, in which TM7 from one CESA can interact with TM6 from a neighboring CESA. Thus, isoxaben might target the interaction interface between TM6 in CESA3 and TM7 in CESA6, although further analyses of the CSC structure and CESA interactions will be required to test this hypothesis.

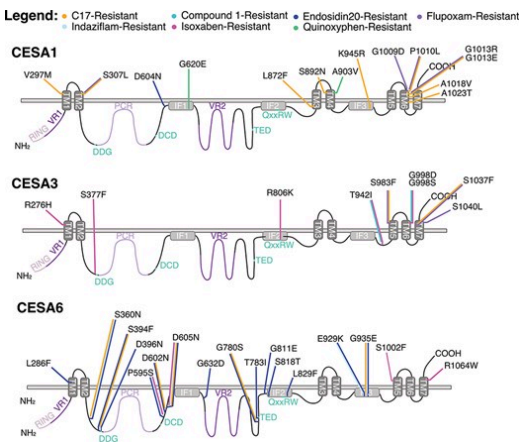


Figure 2: Point mutations conferring CBI resistance in Arabidopsis CESA1, CESA3 and CESA6. Schematic representation of the topology and sequence features *Arabidopsis thaliana* CESA1, CESA3 and CESA6 inspired by Ramírez-Rodríguez and McFarlane (2021). CBI resistance mutation positions are indicated, and CBI resistance is indicated by line colors; mutations that confer resistance to multiple CBIs are indicated by multicolored lines. Key catalytic/coordinating residues are indicated in green: DDG, DCD, TED and QxxRW. CESA features are indicated in varying shades of purple: cytosolic N-terminal RING domain; VR1 and VR2 (residues not conserved among plant CESAs); PCR (residues conserved among plant and algal CESAs); TM (transmembrane helices); IF (cytosolic interface helices), extracellular C-terminus.

Isoxaben-resistant mutants affecting *CESA3* were also resistant to the thiazolidinone carbamate known as Compound 1, suggesting that Compound 1 has a similar mode of action as isoxaben. Interestingly, and opposite to isoxaben, assays of 3H-glucose incorporation into cellulose demonstrated that monocot grasses, such as *Zea* mays were several orders of magnitude more sensitive to Compound 1 than dicots, suggesting that comparative analyses of isoxaben and Compound 1 might provide insight into the structure and evolution of the CSC across different species.

Paper 6: Plant Biochemistry

Question 1

Question: In the exploration of cellulose biosynthesis inhibitors (CBIs) and their multifaceted roles, the paper delves into the intricate balance between fundamental research and applied agricultural practices. Consider the following: The dual role of CBIs, as shown in the paper, involves:

a.) Unraveling the complexities of plant growth and development, while simultaneously serving as potent herbicides for weed control.

b.) Facilitating cellulose biosynthesis to enhance mechanical support in plant cells and promoting sustainable biofuel feedstock.

c.) Inhibiting cellulose biosynthesis and promoting textile production.

d.) Accelerating biofuel feedstock development and limiting paper production.

Question 2

Question: The comprehensive overview of plant cell wall structure and cellulose biosynthesis in the presented passage reveals intricate details about the organization, synthesis, and regulation of cellulose. Consider the highlighted information: The assembly and dynamics of cellulose synthase complexes (CSCs) play a crucial role in cellulose microfibril formation. Which of the following statements accurately describes aspects of CSC assembly and function, as discussed in the passage?

a.) CESAs in Arabidopsis, such as CESA1, CESA3, and one of the CESA6-like clade members, are exclusively responsible for secondary cell wall synthesis.

b.) CSC movement is primarily influenced by cellulose microfibril alignment, and glucose addition is decoupled from glucan chain translocation.

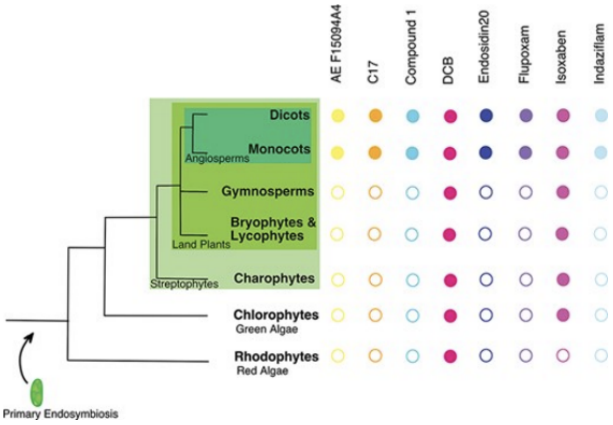
c.) CSCs exhibit 6-fold symmetry, representing six CESA trimers per CSC, and their movement is guided by microtubules for optimal cellulose synthesis.

d.) The plant-specific regions, including N-terminal variable region (VR1), plant-conserved region (PCR), and highly variable region (VR2), are not crucial for CSC assembly and interactions with accessory proteins.

Question 3

Question: Given the description of the visual representation of the breadth of cellulose biosynthesis inhibitor (CBI) studies across Archaeplastida plants and algae, consider the following question:

The phylogenetic representation depicts the testing of CBIs on various land plants and algae within the Archaeplastida supergroup. Which of the following statements accurately reflects the information conveyed in the figure above?



a.) A filled circle indicates that CBIs have been tested on all species within the taxon, providing comprehensive data for that particular group.

b.) An open circle signifies that no CBIs have been developed or tested for the indicated taxon, leading to a lack of research within that specific group.

c.) The exclusion of CBIs tested only on dicots suggests a focus on monocots, ensuring a broad representation of plant diversity in the figure.

d.) The presence of both filled and open circles conveys variability in the extent of CBI testing across different taxa, with some groups receiving more research attention than others.

Question 4

Question: The passage provides an extensive examination of isoxaben, a pre-emergence herbicide known for its potency as a cellulose biosynthesis inhibitor (CBI). Considering the following information presented: Isoxaben exhibits selective activity against various Archaeplastida species, with notable resistance observed in certain grasses. Which of the following statements accurately reflects the factors contributing to isoxaben resistance and its effects on cellulose synthesis?

a.) Grasses resistant to isoxaben typically display increased isoxaben uptake and metabolism, leading to reduced herbicidal effects.

b.) Isoxaben resistance in grasses is associated with a unique cell wall composition, often characterized by the presence of mixed-linkage glucan (MLG) in addition to cellulose.

c.) Isoxaben-induced effects on primary and secondary cell wall biosynthesis are consistent across different plant species, indicating a universal response to the herbicide.

d.) The resistance of Arabidopsis mutants to isoxaben suggests that isoxaben primarily targets CESA1 and CESA3, leading to mutations in these genes.