Biosynthesis of Cellulose and Other (1→4)-β-Glycans
Diversity in cellulose synthase complexes
What is the cellulose synthase complex?

The six-membered hexagonal rosettes are on the inner leaf of the plasma membrane. The impressions of cellulose microfibrils at the exterior each terminate at each rosette.

Rosettes are about 25 nm in diameter, but the cytosolic catalytic domains below these membrane components occupy a much larger diameter.

Hypothetical model of conformation for a single CesA protein subunit. The eight transmembrane helices form a pore through which a single β-glucan chain is secreted. Each CesA subunit must interact with other such subunits to form the synthase complex. The Zn-fingers, P-CR and CSR are potential interaction sites.

Based on calculations of the rate of cellulose synthesis, the number of microfibrils, and number of rosettes, Schneider and Herth estimated that the time required to synthesize a microfibril is about 10 min.


Haigler and Brown demonstrated that rosettes were continuously produced in the Golgi membranes of Zinnia cells during tracheary element development, suggesting continuous turnover and begging the question of whether one rosette makes one microfibril.

They observed the fluorescence of YFP:CesA6 appeared and disappeared rapidly from the plasma membrane, and that isoxaben, a sensitive and specific inhibitor of cellulose, causes rapid loss of CesA from the plasma membrane particles.


They showed that prolonged DCB treatment of protoplasts resulted in enhanced cellulose synthesis, with rates nearly 3-fold higher than controls, when the DCB was removed. These experiments indicate that the CesAs do not turn over from the membrane if cellulose microfibrils are not made.
The cotton fiber zinc-binding domain of cellulose synthase A1 from *Gossypium hirsutum* displays rapid turnover *in vitro* and *in vivo*

Debora Jacob-Wilk†, Isaac Kurek‡, Patrick Hogan, and Deborah P. Delmer§

Section of Plant Biology, One Shields Avenue, University of California, Davis, CA 95616


- The half-life of the reduced monomer of GhCesA1 ZnBD is less than 30 min in vivo, far less than the average membrane protein

- In *vitro* degradation of the reduced GhCesA1 ZnBD is inhibited by the proteosome inhibitor MG132, but also by E64 and EGTA, suggesting initiation of proteolysis by cysteine protease activity rather than the proteosome

- They identified by a yeast two-hybrid system a metallothionein that interacts with GhCesA1 ZnBD

- They propose a model wherein active cellulose synthase complexes contain CesAs in a dimerized form, and that turnover and degradation are mediated through reductive zinc insertion by metallothionein and subsequent proteolysis involving a cysteine protease
Fig. 1. Half-life of GhCesA1 in 24-DPA cultured cotton fibers. Fibers, with their associated ovules, were incubated for the indicated times in the presence or absence of 400 μM cycloheximide (CHX). Western blot analysis of total membrane proteins (5 μg per lane) was carried out by using antibody against the GhCesA1 ZnBD (Top) and Pm-SuSy (Middle). Total membrane proteins (20 μg per lane) were separated by SDS/PAGE and detected by silver staining (Bottom).
Fig. 6. Effect of H$_2$O$_2$ on the phenotype of Arabidopsis seedlings treated with CGA 325'615. Four-day seedlings were analyzed prior to (A) and after (B) treatment with CGA 325'615 in the presence of H$_2$O$_2$ concentrations as indicated. To recover the CGA 325'615 phenotype, treated seedlings were further incubated for additional 14 hr in the presence of herbicide (C).
How do plants make cellulose and other \((1\rightarrow4)-\beta\text{-glycans}\)?

(i.e. what is the catalytic mechanism?)

Herth, 1985

After Delmer, 1999
Three problems/questions with the protein model:

1. Is a membrane channel of only 8 membrane spanning domains sufficient to allow a glucan chain to be extruded?

2. If CesAs are recruited into the rosette via the Zn-finger domains, what happens after two couple?

3. How can a \((1\rightarrow4)\)-\(\beta\)-glucosyl linkage, in which every sugar is rotated 180° with respect to each neighbor, be made processively in a single catalytic site, if one sugar is added at a time to the acceptor end.
Iterative inversion of a single non-reducing terminal sugars alternates the O-3 and O-4 hydroxyl as acceptor.

The true catalytic unit could be a dimer that generates cellobiose units.
Is a membrane channel of only 8 membrane spanning domains sufficient to allow a glucan chain to be extruded?

All prokaryotic and plant hexose and maltose transporters contain from 12 to 18 membrane spanning domains.

(Sherson et al., 2000; Klepek et al., 2009)

Plant callose synthases are roughly twice the size of CesAs and contain 16 membrane spanning domains, i.e. twice those of CesAs

(Doblin et al., 2001; Hong et al., 2001)

From Hong Lab: http://www.cals.uidaho.edu/mmbb/zhong
Arguments against the two-site model: The SpsA synthase

Only one UDP-Mg\(^2+\) site accommodated in the protein

(Charnock and Davies, 1999)

A structural homolog of the SpsA synthase from Bacteroides fragilis crystallizes as a dimer preserving both UDP binding centers

(Palani et al.: http://www.pdb.org/pdb/explore/explore.do?structureId=3BCV)
Arguments against the two-site model: Hyaluronan synthase

Both binding sites for the nucleotide sugar substrates and transferase activities are in a single polypeptide

(DeAngelis and Weigel, 1994; Jing and DeAngelis, 2000)

However, when host cells harboring constructs with Class II HA synthases, in which each site is independently disrupted, they are still able to make HA.

Thus, a direct interaction of two synthases is inferred for complementation HA synthesis.

(Jing and DeAngelis, 2000; Weigel and DeAngelis, 2007)
Catherine Rayon
Recombinant CesA-CatD from expression in *E. coli* forms higher order aggregates

Monomeric form spontaneously dimerizes

Protein directly From Ni-column

Isolated monomer rerun

Isolated monomer concentrated 5x and rerun

*Subhangi Ghosh*

*Anna Olek*
Recombinant CesA ZnF domains dimerize in a redox-dependant manner

Anna Olek
The Class-Specific Regions (CSR) have conserved Cys residues

Vergara and Carpita, 2001
Behavior of recombinant CesA CatD and ZnF-CatD proteins in analytical centrifugation

CatD only

control

monomer
dimer

+ 10mM DTT

ZnF-CatD

Lake Paul
Three problems are solved by the catalytic dimer model:

1. A channel of $8 \times 2 = 16$ membrane spanning domains would be equivalent to callose synthase and most sugar transport proteins.

2. The synthesis of cellobiose units eliminates the steric problem of iterative synthesis of a single unit because the O-4 would always be in the same location in the non-reducing end of the growing chain.

3. The dimer produces two Zn-finger domains to recruit into larger complexes.
Some questions...

*Are certain heterodimers favored?*
We are going to address this using Multicolor Bimolecular Fluorescence Complementation (MBiFC) *in vitro* and *in vivo.*

*Do the Zn-finger domains recruit dimers into larger complexes in a redox-dependent manner?*
'Daisy-chaining' seems to be working, but the estimated sizes from analytical centrifugation are not predictable. Still need to prove redox dependancy. We are also employing AFM to see what they look like.

*How many UDP-Glc are bound per dimer?*
We will use $[\alpha^{-32P}]$UDP plus Mg$^{2+}$ to determine the stoichiometry.

*Can we crystallize the most stable dimer or reduced monomer???
Formation of catalytic dimers would require a revision of the particle rosette structure.

(...but then how the three-way connections get made?)
ER Synthesis: Cell wall proteins: HRGP, GRP, PRP, AGP

Enzymes: Hydrolases, Esterases, Peroxidases, Polysaccharide synthases

Golgi synthesis: Pectins, Xyloglucan, GAX, β-glucan

Glycosylation; Modified Glycoproteins HRGP, AGP

Plasma membrane: Cellulose, Callose
β-Glucans are unbranched polymers of celloextrin oligomers connected by single (1→3)-linkages
The smallest unit is a cellotriose connected by single (1→3)-linkages, and it is the most abundant.

<table>
<thead>
<tr>
<th></th>
<th>Barley*</th>
<th>Oat*</th>
<th>Maize</th>
<th>Lichenin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mole %</td>
<td>70</td>
<td>64</td>
<td>68</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>31</td>
<td>26</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>2.3</td>
<td>3.6</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>1.5</td>
<td>1.7</td>
<td>1.9</td>
</tr>
</tbody>
</table>

A sequence-dependent enzyme digests β-glucan into oligomers that are separated by HPAEC.
β-Glucan synthase is located in the Golgi apparatus.

David Gibeaut
Synthesis of the odd-numbered units exhibits different kinetics from synthesis of even-numbered units.

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Radioactivity per Fraction</th>
<th>Molar Ratio of Paired Oligomers</th>
<th>UDP-Glc Concentration, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>50 µM</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>2.5 µM</td>
<td>1.5</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>2 µM</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
<td>1.5 µM</td>
<td>2.5</td>
<td>30</td>
</tr>
</tbody>
</table>

Marcos Buckeridge
Proteinase K reduces β-glucan synthase in intact Golgi membranes...

...but at low concentrations only the synthesis of the cellotriosyl units is knocked out...

![Graph showing relative activity of IDPase and β-glucan synthase vs. Proteinase K concentration.](image)

- **A**: Graph showing relative activity of IDPase and β-glucan synthase with Proteinase K concentration.
  - IDPase activity remains relatively constant.
  - β-glucan synthase activity decreases as Proteinase K concentration increases.
- **B**: Graph showing relative activity of total β-glucan, trimer, and tetramer vs. Proteinase K concentration.
  - Total β-glucan activity decreases sharply with Proteinase K concentration.
  - Trimer and tetramer activities decrease with Proteinase K concentration, but remain above zero.
How the acquisition of a third mode of glycosyl transfer can convert a cellulose synthase into a mixed-linkage $\beta$-glucan synthase.
β-glucan synthase at the Golgi membrane is the topological equivalent of cellulose synthase plus an accessory glycosyl transferase.
Cellulose synthases
Catherine Rayon - U J-V. Picardie
Anna Olek - Purdue
Subhangi Ghosh - Purdue
Lake Paul - Bindley Bioscience Center
Chris Staiger - Purdue

β-glucan synthases
Marcos Buckeridge - USP/Campinas
Claudia Vergara - Mich State
Breeanna Urbanowicz - CCRC Georgia

siRNAs
Mick Held - Mich State/Ohio Univ
Steve Scofield - USDA-ARS
Amanda Brandt

Cell Wall Biology@Purdue
Maureen McCann
Anna Olek
Bryan Penning
Nathan Hood
Ashley Hudson
John Klimek
Rachelle Buuck
Joe Cox
Danisha Debowles
Ethan Lapham
Rachel Mertz

Tropical Maize & Sweet Sorghum
Fred Below - UIUC
Steve Moose - UIUC

Sylvie Brouder
Dennis Buckmaster
Klein Ileleji
Nathan Mosier
Jeff Volenec
Cliff Weil

IBM/RILs
Bryan Penning
Mick Held - Ohio Univ
Mark Davis - NREL
Rob Sykes - NREL
Ashley Hudson
Nick Babcock
Cliff Weil

EBRC
Purdue University
Discovery Park
C3Bi
USDA
NSF
DOE