Amino acids essential for the assembly of cellulose synthase complexes

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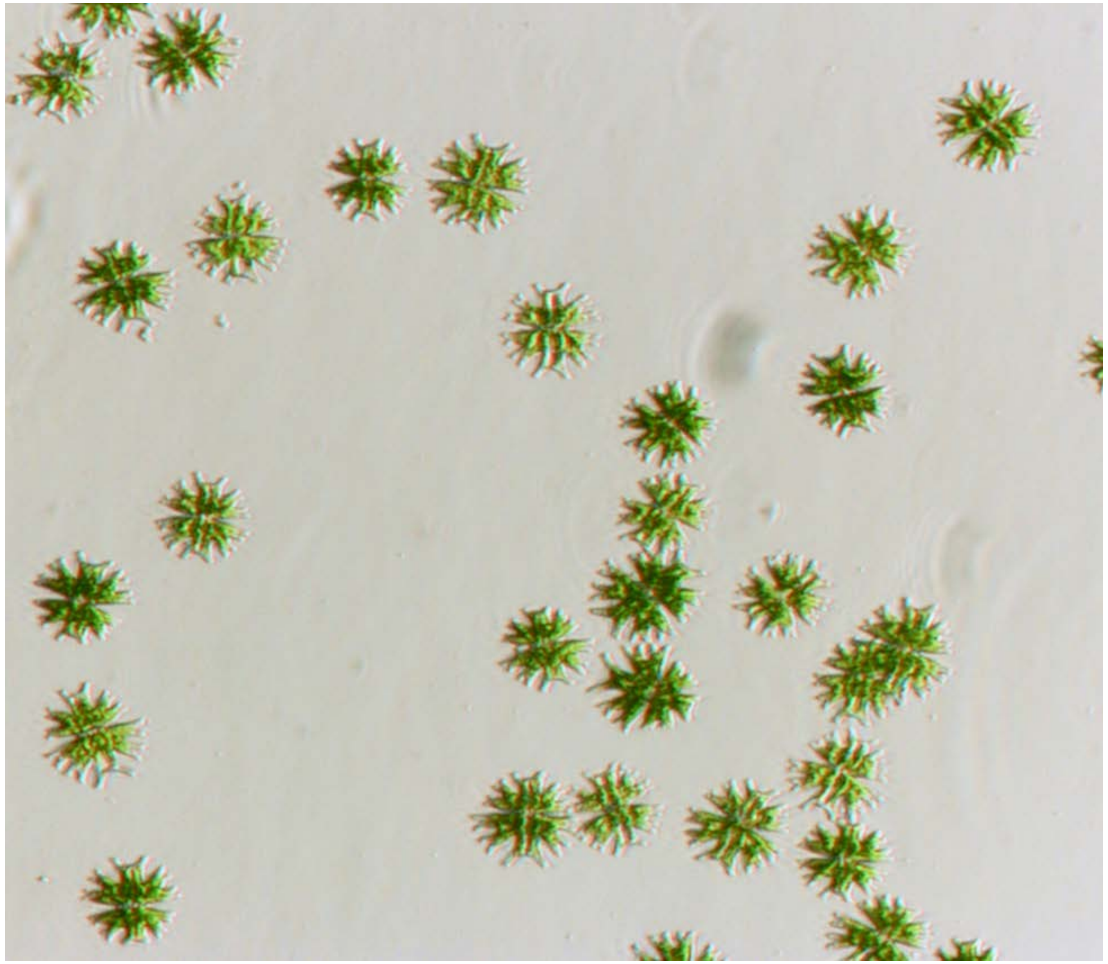
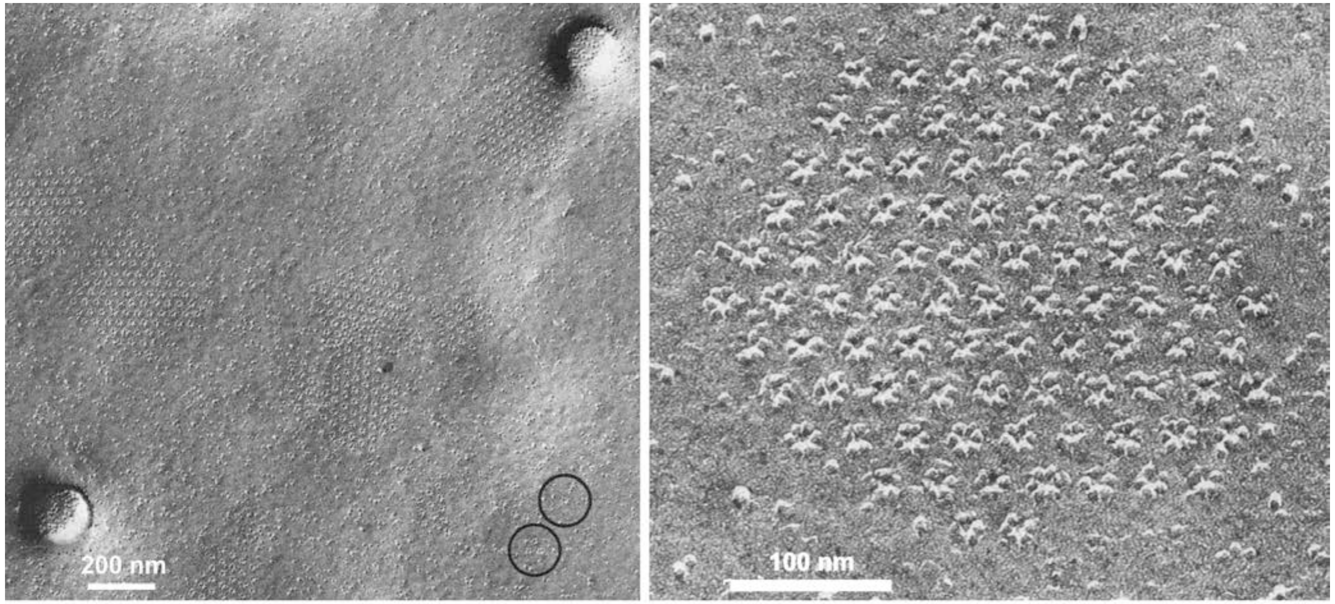
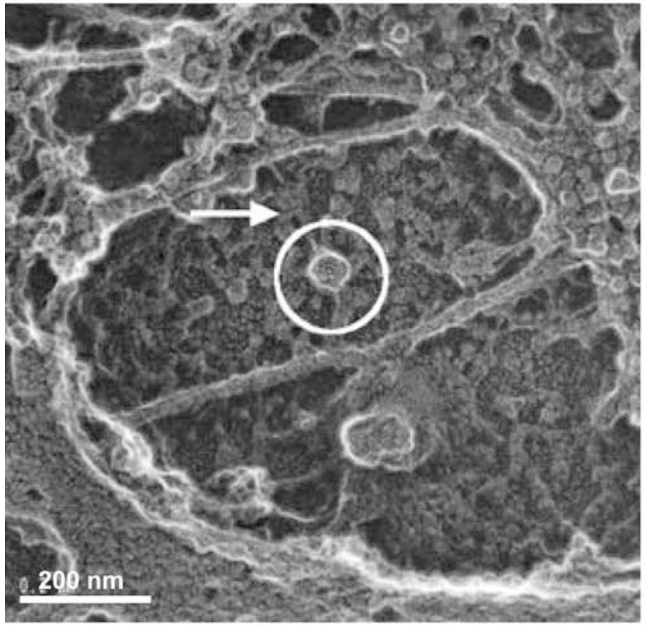
The ‘rosettes’ of Micrasterias



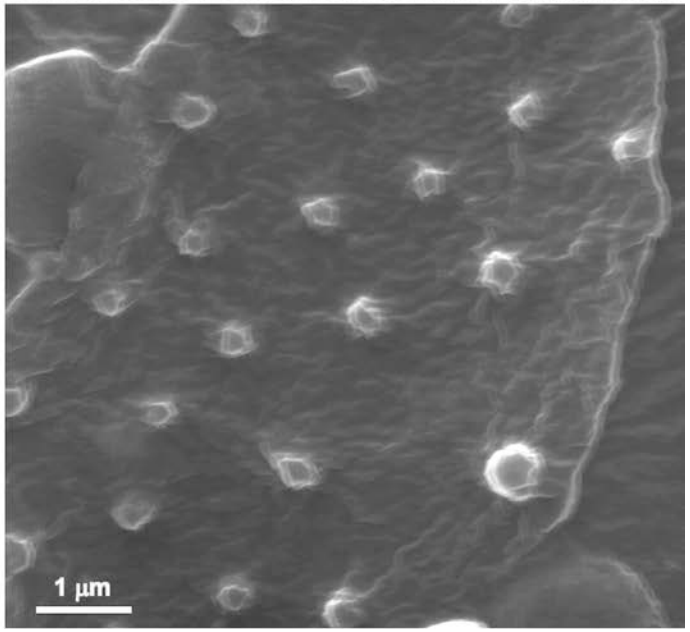
Giddings et al.: “The large circular indentations may correspond to forming slime secretion pore complexes.”

Giddings *et al*. (1980) *J. Cell Sci.* ***84***:327-339



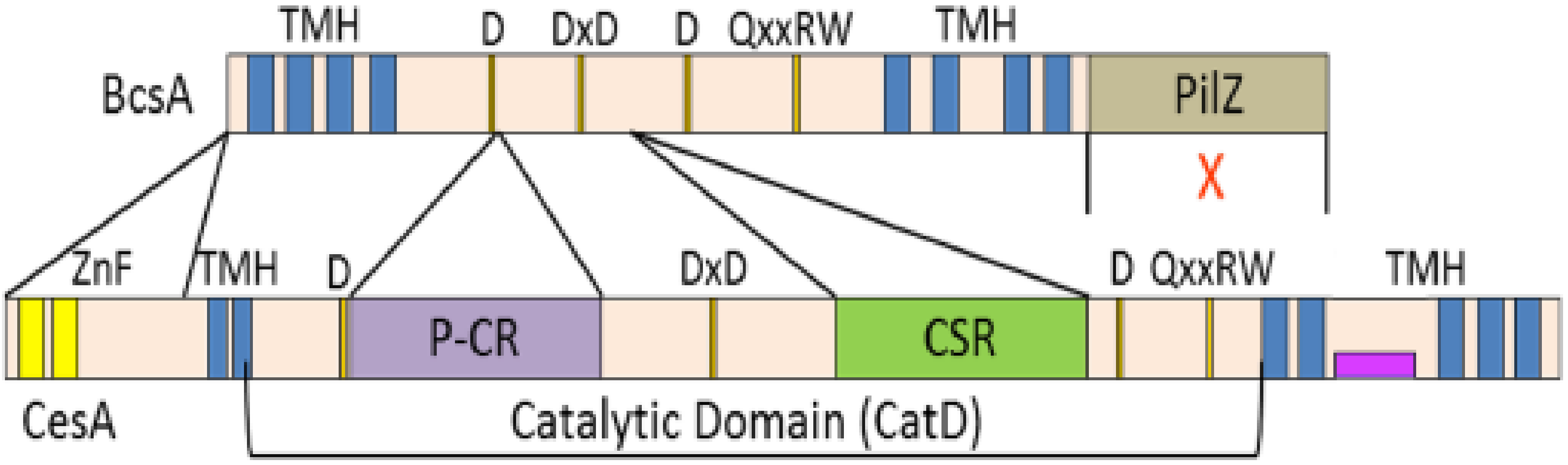


Bowling and Brown (2008)
Protoplasma ***233***:115-127



Donohoe and Carpita, *unpublished*

Plant Cellulose Synthases (CesAs) have three additional sequences



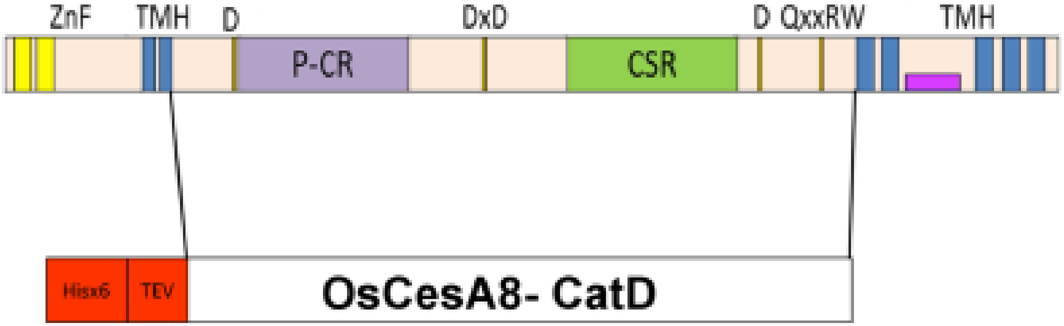
- ***Plant CesAs are about 120-125 kDa with seven trans-membrane domains, with a 57-60 kDa catalytic core sandwiched between TMD2 and TMD3.***

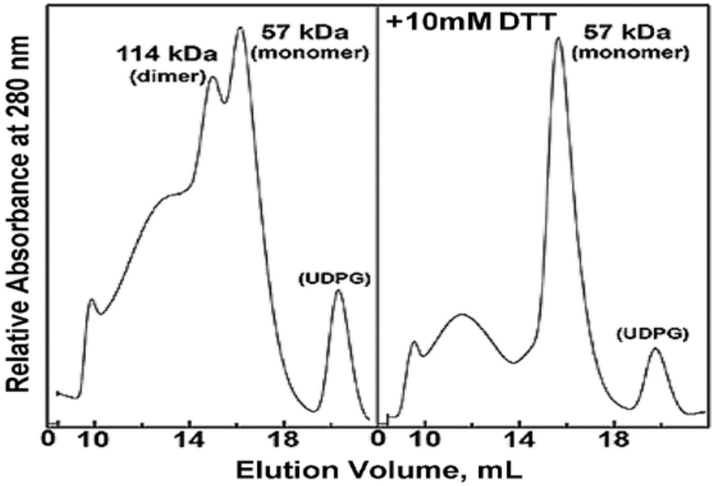
- ***The catalytic core is strongly conserved with bacterial ancestors, sharing four characteristic D, DxD, D, QxxRW catalytic motifs essential for UDP-Glc binding and catalysis***

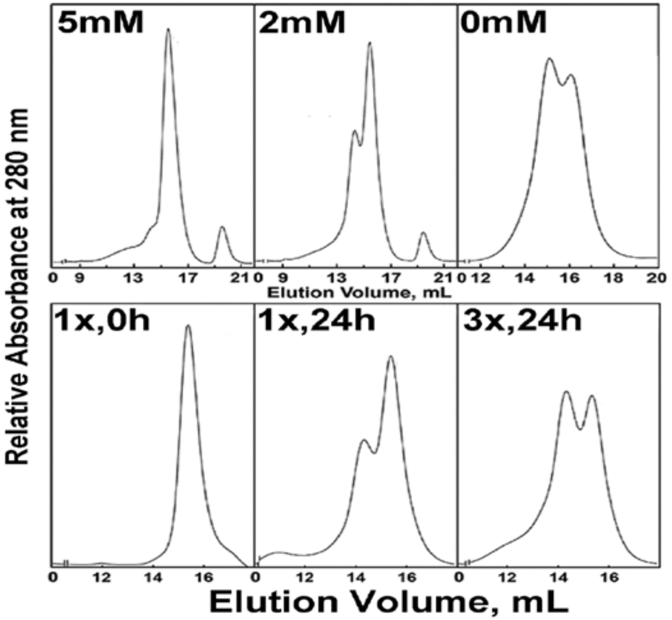
- ***Plant CesAs contain three sequence insertions: a Zn-finger that functions in redox-dependent dimerization, and two insertions, the P-CR and CSR, in the catalytic core.***

- ***Plant CesAs lack the PilZ regulatory domain of bacterial synthases***

The soluble rice CesA8-CatD domains dimerize in a redox-dependent manner



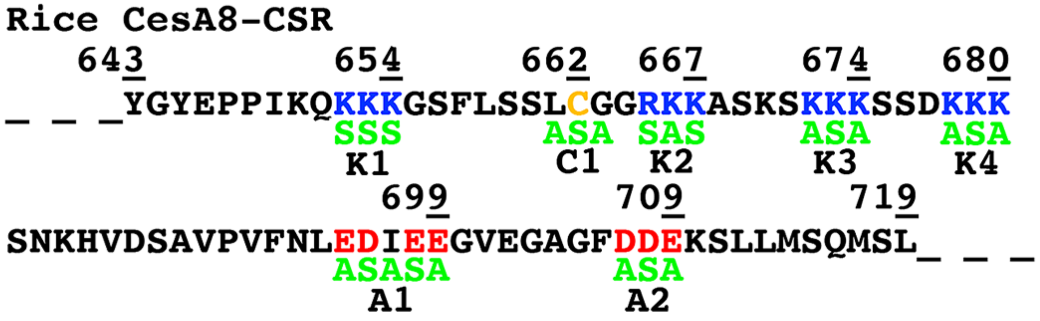


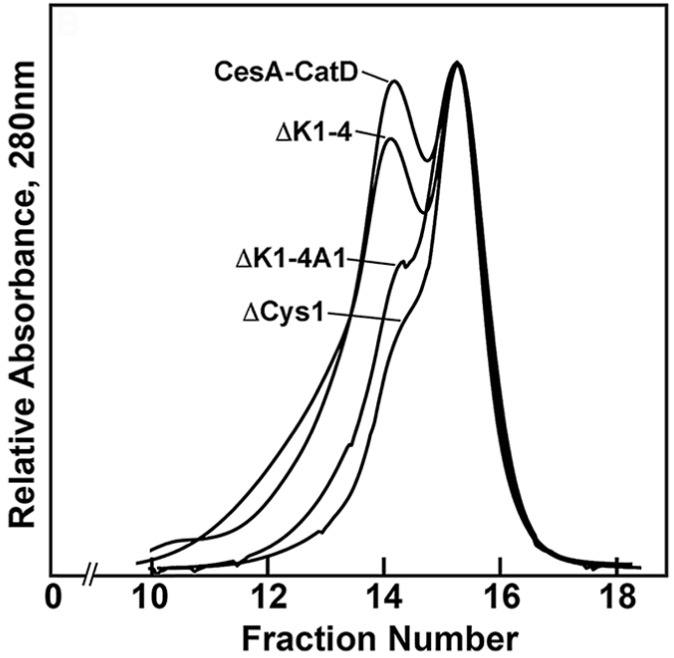


- ***The soluble recombinant 57 kDa catalytic domain (CatD) within the CesA (between TMD 2 and 3) is a two-domain structure.***
- ***The soluble recombinant protein as a monomer when DTT is included in the extraction buffers***
- ***Dilution of the DTT or concentration of the protein by spin-filtration results in dimerization.***

Olek, Rayon *et al.* (2014) *Plant Cell* **26**:2996–3009

The CSR has conserved basic, acidic, and C motifs; we mutated several of these to determine their impact on dimerization

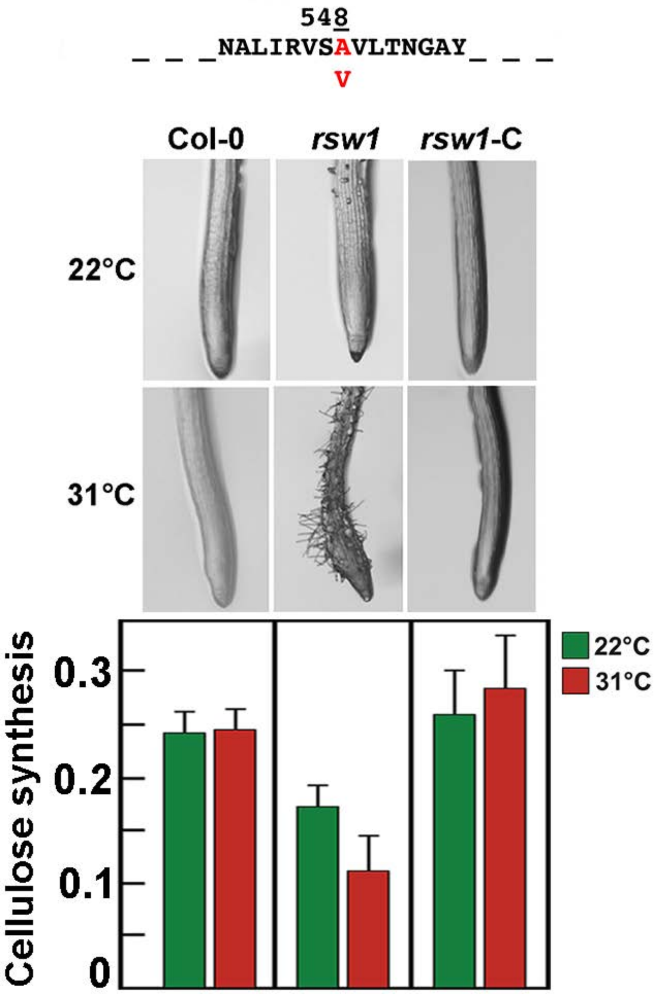


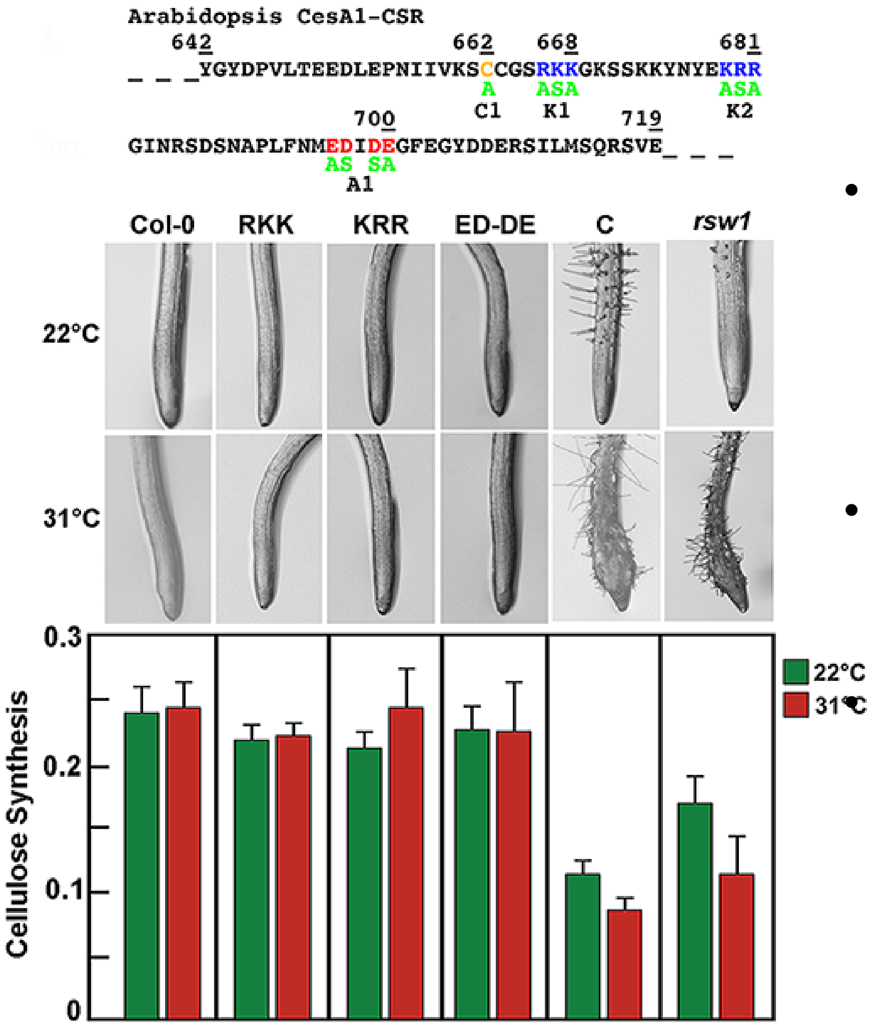


- *Substitution of all four basic amino-acid triplets with Ala or Ser had little impact on dimerization.*
- *Substitution of the acidic motif in addition to the four basic motifs reduced but did not eliminate dimerization.*
- *Substitution of the Cys alone essentially eliminated dimerization.*

Olek *et al*. (2023) *Plant Physiol.* **191**:142-160

We used the temperature-sensitive *root-swelling1* (*rsw1*) as a testbed for complementation by mutated CSR motifs in ΔCesA1 constructs in transgenic Arabidopsis





- ***Substitution of either of the two basic amino-acid triplets with Ala or Ser had no impact on complementation of the rsw1 phenotype to wild type.***

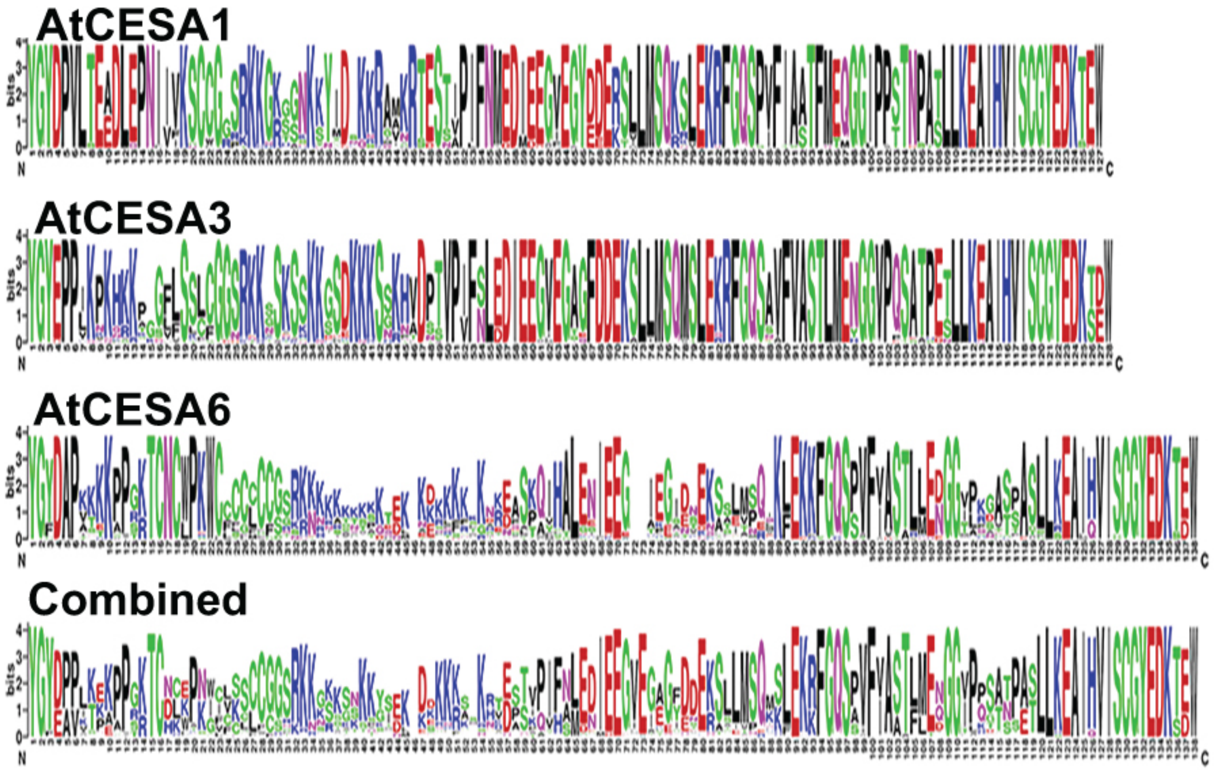
- ***Substitution of the acidic motif had no impact on complementation***

- ***Substitution of the Cys prevented complementation of the rsw1 phenotype***

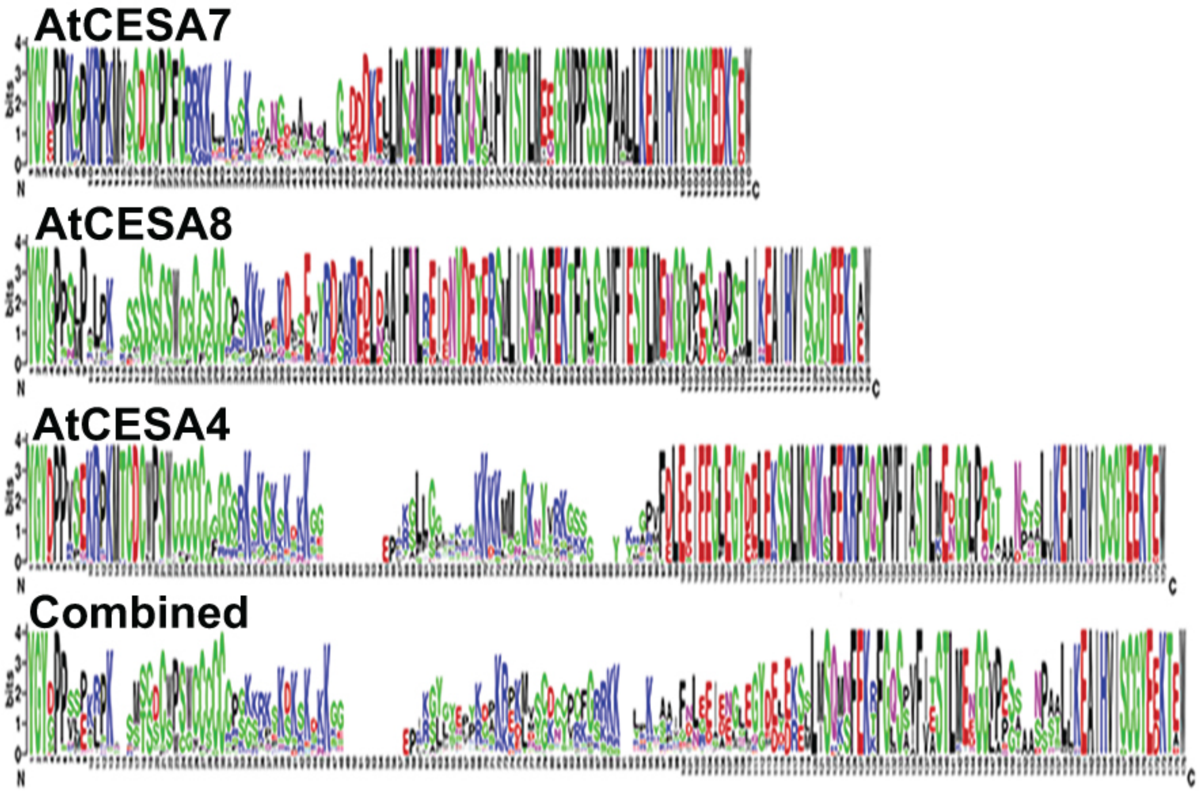
Olek *et al*. (2023) *Plant Physiol.* **191**:142-160

The CSR is characterized as a sequence of Intrinsic Disorder; however, the CSRs of both the primary wall and secondary wall CesAs show remarkable conservation and differ in the degree of disorder

Primary wall CESAs



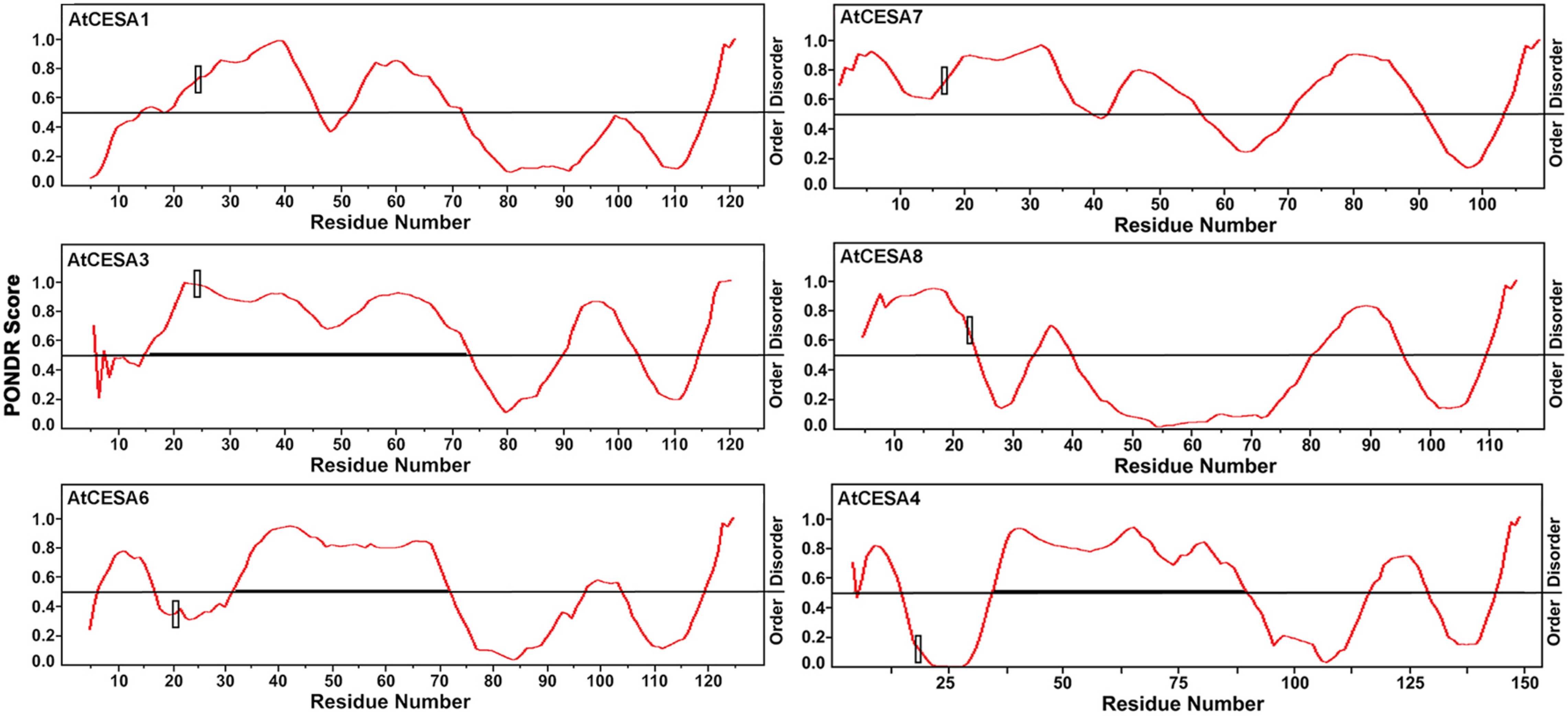
Secondary wall CESAs



WebLogos show strong conservation of sequence among 33 diverse species of angiosperms; diversity is greater in CESA6 and CESA4 and narrower in CESA1 and CesA3, and in CESA7 and CESA8

Olek *et al.* (2023) *Plant Physiol.* **191**:142-160

PONDR plots show relative order and disorder vary among classes

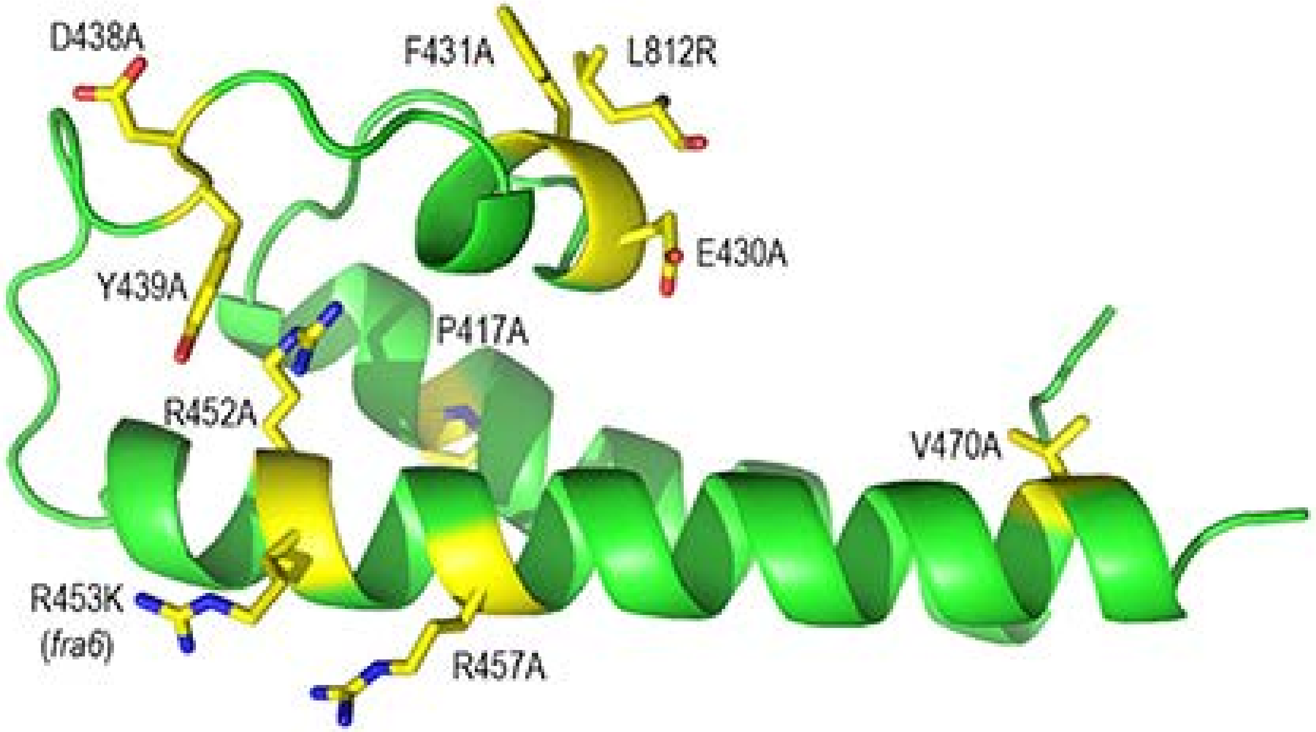


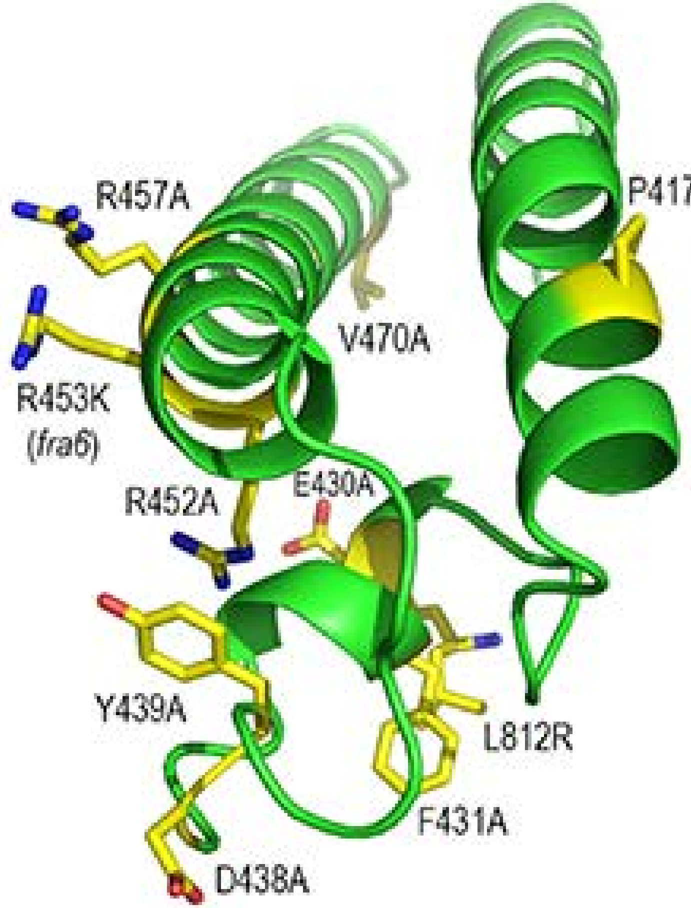
The position of essential Cys residue is independent of degree of disorder; the more variable CSRs of CESA6 and CESA4 give more similar PONDR plots

Olek *et al*. (2023) *Plant Physiol.* **191**:142-160

P-CR crystal structure of the rice CESA8 has a coiled-coil domain with a Pro_{417}-induced ‘kink’ and large connecting loop

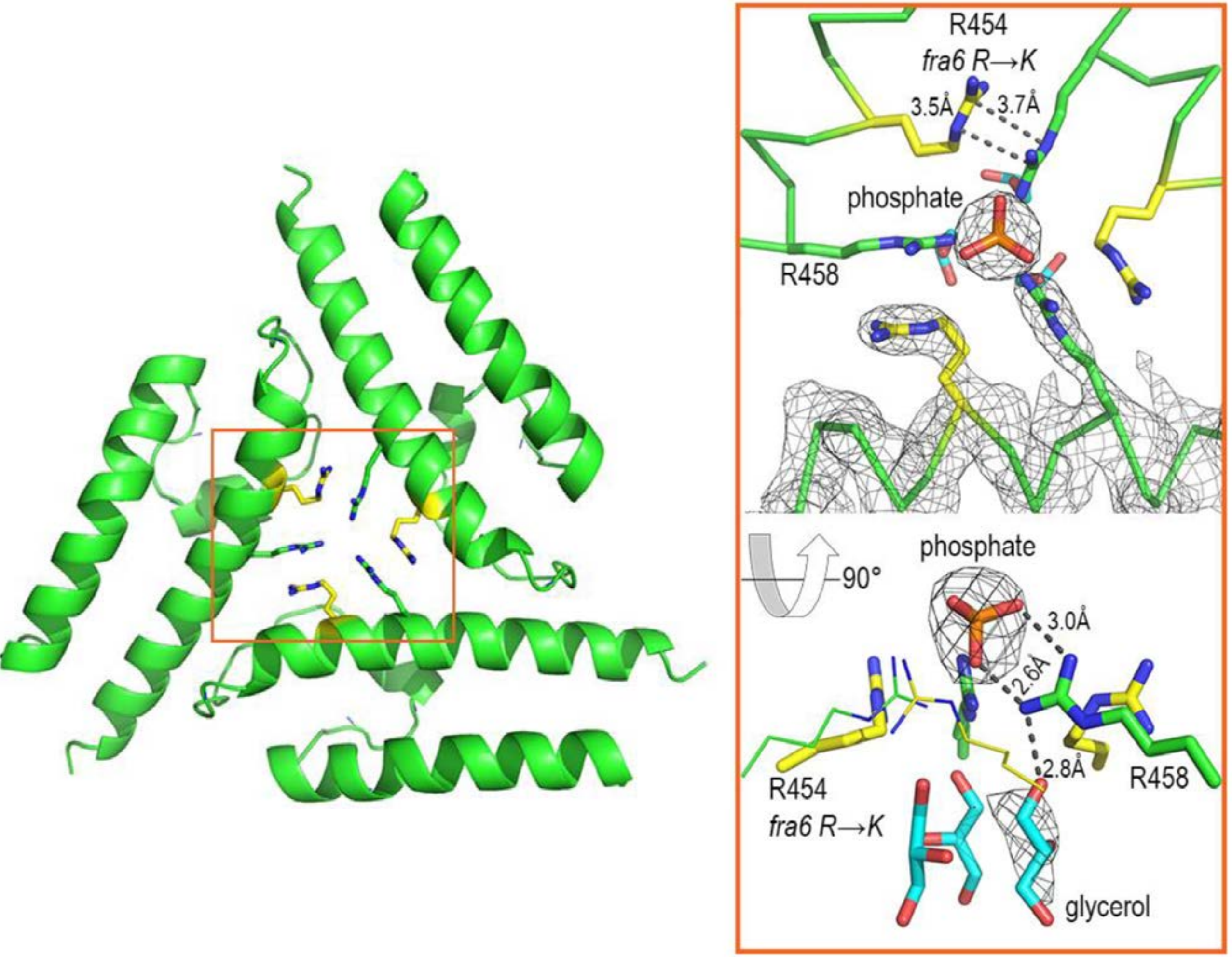
We made mutations in the P-CR of Arabidopsis CesA1 to test their essential function



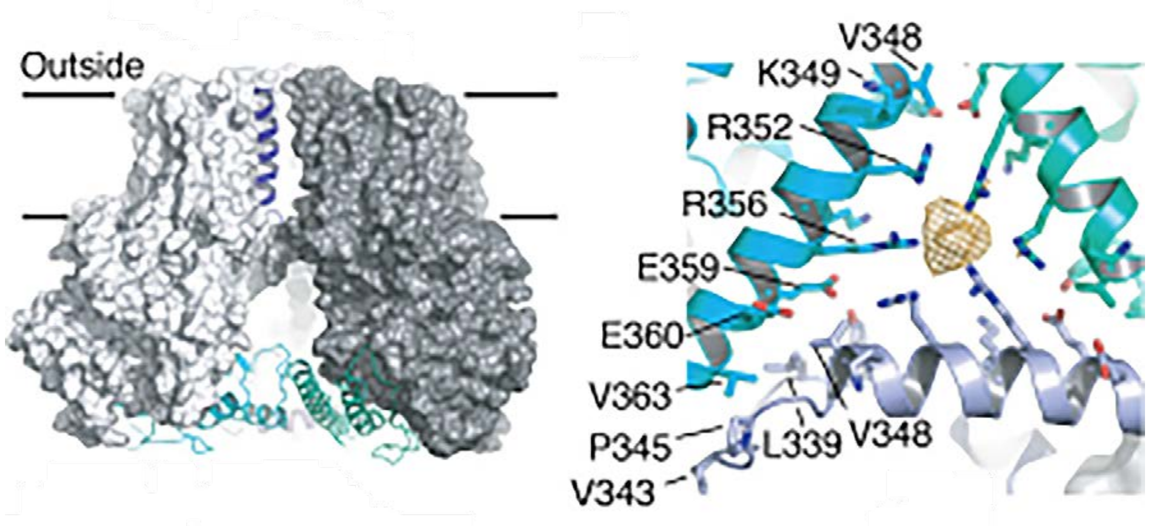


Olek *et al*. (2023) *Plant Physiol.* **191**:142-160

A P-CR trimer forms in the crystal structure by coordinating three rice Arg458 residues by a phosphate residue; three cognate Arg do the same in a recombinant Ptt CesA8

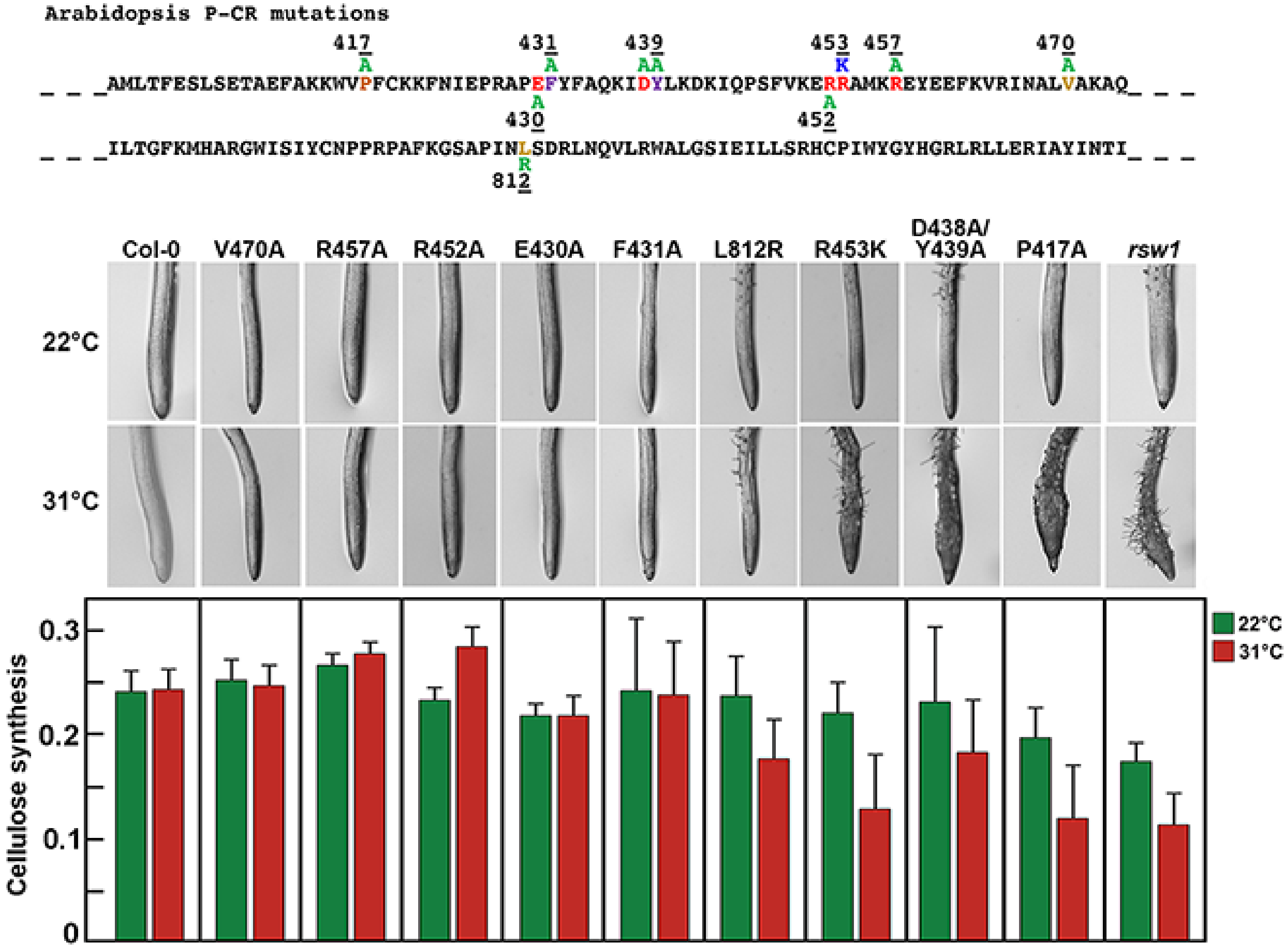


Rushton *et al.* (2017) *Plant Physiol.* **173**: 482–494

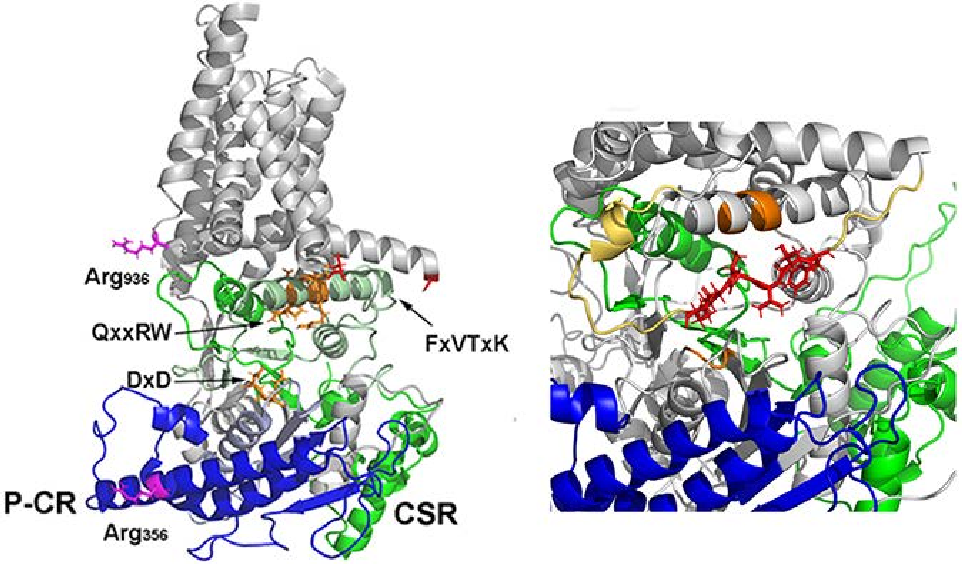


Purushotham *et al.* (2020) *Science* **369**: 1089-1094

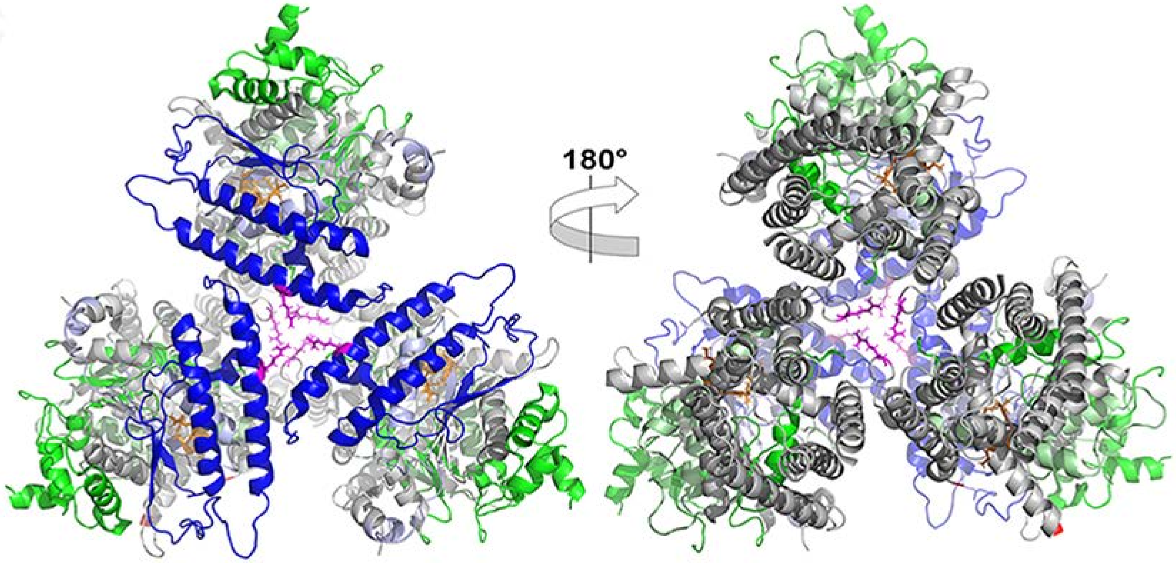
AtCesA1 constructs with mutated Arg_{457} residues in the P-CR fully complement *rsw1*



Olek *et al*. (2023) *Plant Physiol.* **191**:142-160



PttCESA8



PttCESA8 trimer

Summary

A specific Cys residue in the CSR is essential for dimerization of CesA monomers, and this dimerization is essential for CesA assembly and function

Although the CSR has regions of intrinsic disorder, CesAs of different classes show display diversity

The Arg$_{457}$ of AtCESA1 coiled-coil domain of the P-CR is not essential for assembly of trimers or function. An alternative Arg$_{936}$ in the C-terminal domain might substitute

AlphaFold modeling of the conserved FxVTxK motif, involved in substrate ‘gating’ in the BscA synthase, aligns it over the QxxRW catalytic sequence. A flexible C-terminus of the P-CR is a candidate for interaction

Olek *et al*. (2023) *Plant Physiol.* **191**:142-160

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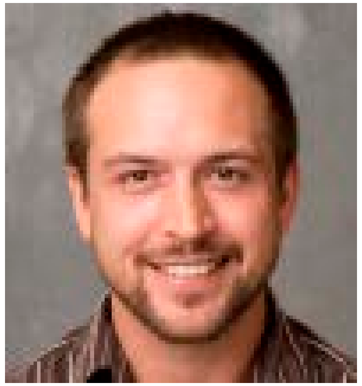
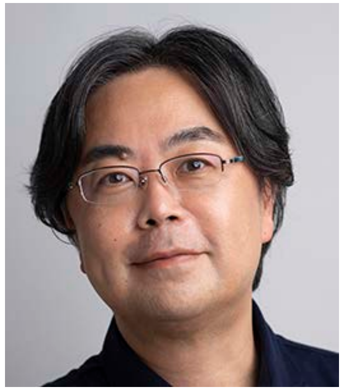

Phillip Rushton
Purdue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