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4 authors, including:

Cristina Vettori

Italian National Research …

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Editors

Cristina Vettori
Institute of Bioscience and BioResouces
National Research Council
Sesto Fiorentino (FI)
Italy

Giovanni Giuseppe Vendramin
Institute of Bioscience and BioResouces
National Research Council
Sesto Fiorentino (FI)
Italy

Donatella Paffetti
Department of Agriculture, Food and Environmental Sciences
University of Florence
Florence
Italy

Davide Travaglini
Department of Agricultural, Food and Forestry Systems
University of Florence
Florence
Italy

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Discovery of the biological role of PHENYLCOUMARAN BENZYLIC ETHER REDUCTASE (PCBER), one of the most abundant proteins in poplar xylem

Claudiu Niculaes[a,b,c], Kris Morreel[a,b], Hoon Kim[d], Fachuang Lu[d], Lauren McKee[e], Bartel Vanholme[a,b], Riet De Rycke[a,b], Magnus Hertzberg[f], Vincent Bulone[e], Andrea Polle[g], John Ralph[d] and Wout Boerjan[a,b]

[a] Department of Plant Systems Biology, VIB, 9052 Ghent, Belgium.

[b] Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium.

[c] Current address: Lehrstuhl für Genetik, Wissenschaftszentrum Weihenstephan, Technische Universität München, 85354 Freising, Germany

[d] Department of Biochemistry and the DOE Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, University of Wisconsin-Madison, Madison, WI 53726, USA.

[e] Division of Glycoscience, School of Biotechnology, Royal Institute of Technology (KTH), AlbaNova University Centre, 106 91 Stockholm, Sweden

[f] SweTree Technologies AB, P.O Box 4095, SE-904 03 Umeå, Sweden

[g] Forstbotanik und Baumphysiologie, Büsgen-Institut, Georg-August-Universität Göttingen, Göttingen, Germany

Claudiu Niculaes niculaes@wzw.tum.de

Kris Morreel krmor@psb.vib-ugent.be

Hoon Kim hoonkim@wisc.edu

Fachuang Lu fachuanglu@wisc.edu

Lauren McKee mckee@kth.se

Bartel vanholme bahol@psb.vib-ugent.be

Riet De Rycke riryc@ugent.be

Magnus Hertzberg Magnus.Hertzberg@SweTree.com

Vincent Bulone bulone@kth.se

Andrea Polle apolle@gwdg.de

John Ralp Jralph@wisc.edu

Wout Boerjan woboe@psb.vib-ugent.be

Background

Wood is the most abundant renewable natural material on earth and a prime source for timber, pulp and paper. In addition to these traditional sectors, wood is increasingly considered as an alternative for the production of second generation biofuels such as bioethanol. Given the worldwide importance of wood, it is striking that the biological role of one of the most prominent proteins in poplar xylem, Phenylcoumaran Benzylic Ether Reductase (PCBER), has remained obscure. PCBER was discovered 15 years ago as one of the most abundant proteins in 2-dimensional gels made from poplar xylem (Vander Mijnsbrugge 2000a; 2000b). PCBER belongs to a large class of NADPH dependent oxido-reductases. Davin et al (1999) showed that both the poplar and the pine PCBER can reduce dehydrodiconiferyl alchol (DDC) *in vitro*. DDC is a dimer of coniferyl alcohol, one of the main lignin monomers. Given that PCBER is a cytoplasmic protein and that monolignol coupling and further polymerization to lignin are believed to occur in the cell wall, the question raised what the *in vivo* substrate of PCBER could be, why PCBER is so abundant, and what its biological role is.

Material and methods

Transgenic poplars (*Populus tremula x P. tremuloides*) were made that were downregulated for PCBER by an RNAi approach. Young developing xylem was scraped (~0.5 cm deep) with a scalpel while frozen, from 40 cm long debarked stems harvested from the greenhouse grown poplars. The material was then ground to a fine powder in a mortar cooled with liquid nitrogen, and immediately processed or stored at -70 °C. Immunodetection was according to standard procedures using a polyclonal antibody raised against recombinant poplar PCBER (Vander Mijnsbrugge et al., 2000b). Phenolic profiling was done according to Niculaes et al. (2014). PCBER enzymes used for enzyme activity tests were heterologous produced by PSF (http://www.vib.be/en/research/services/Protein-production-purification-and-analysis/Pages/default.aspx).

Results and conclusion

Transgenic poplars downregulated for PCBER by an RNAi approach were first analyzed by Western blotting, which revealed the strong downregulation of PCBER protein abundance. Lignin amount and composition, as analyzed by acetylbromide, thioacidolysis and NMR, and cellulose amount, were similar between transgenic and wild type trees, indicating that PCBER had no major role in cell wall biosynthesis. Comparative metabolite profiling of the transgenic poplars downregulated for *PCBER* revealed the *in vivo* substrate and product of PCBER. Based on mass spectrometry and NMR data, the substrate was identified as a hexosylated 8–5-coupling product between sinapyl alcohol and guaiacylglycerol and the product as its benzyl-reduced form (Figure 1). This activity was confirmed *in vitro* using a purified recombinant PCBER expressed in *Escherichia coli*. Assays performed on 20 synthetic substrate analogs revealed the enzyme specificity. In addition, the xylem of *PCBER*-downregulated trees accumulated over 2000-fold higher levels of cysteine adducts of monolignol dimers. These

compounds could be generated *in vitro* by simple oxidative coupling assays involving monolignols and cysteine. From these data we deduce that apparently, some of the monolignols that are biosynthesized in the cytoplasm are oxidized to radicals in the cytoplasm, upon which they spontaneously couple to form dimers (and higher order oligolignols). The coumaran type of dimers are substrates for PCBER and are reduced to molecules that become themselves radical scavengers. In the absence of PCBER, the cell makes less of the reduced dimers (thus less scavengers) and the cytoplasm accumulates products derived from the increased oxidative conditions in the cytoplasm. Thus, the data suggest that the function of PCBER is to reduce phenylpropanoid dimers *in planta* to form antioxidants that protect the plant against the oxidative damage associated with lignifying cells. In addition to unveiling the catalytic activity of one of the most abundant enzymes in wood, our data provide the first experimental evidence for an *in planta* antioxidant role of a monolignol coupling product.

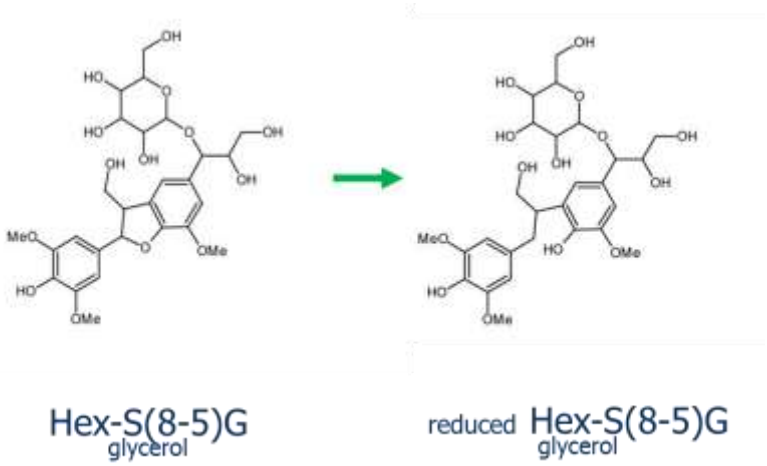

Figure 1. *In vivo* substrate and product of PCBER

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