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Pseudo half-molecules of the ABC transporter, COMATOSE, bind Pex19 and target to peroxisomes independently but are both required for activity

Yvonne Nyathi [a,1,2], Xuebin Zhang [b,1,3], Jocelyn M. Baldwin [c], Kristin Bernhardt [b,d], Barbara Johnson [a], Stephen A. Baldwin [c], Frederica L. Theodoulou [b,*], Alison Baker [a]

[a] Centre for Plant Sciences, Irene Manton Building, University of Leeds, Leeds LS2 9JT, UK
[b] Biological Chemistry and Crop Protection Department, Rothamsted Research, Harpenden AL5 2JQ, UK
[c] Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK
[d] Institut für Biochemie der Pflanzen, Heinrich-Heine Universität Düsseldorf, 40225 Düsseldorf, Germany

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ABSTRACT

Peroxisomal ABC transporters of animals and fungi are "half-size" proteins which dimerise to form a functional transporter. However, peroxisomal ABC transporters of land plants are synthesised as a single polypeptide which represents a fused heterodimer. The N- and C-terminal pseudo-halves of COMATOSE (CTS; AtABCD1) were expressed as separate polypeptides which bound Pex19 in vitro and targeted independently to the peroxisome membrane in yeast, where they were stable but not functional. When co-expressed, the pseudo-halves were fully functional as indicated by ATPase activity and rescue of the *pxa1pxa2Δ* mutant for growth on oleate. The functional significance of heterodimer asymmetry is discussed.

Structured summary of protein interactions:
PEX19-1 binds to **CTS-N** by pull down (View Interaction)
PEX19-1 binds to **CTS-C** by pull down (View Interaction)
PEX19-2 binds to **CTS-N** by pull down (View Interaction)
PEX19-2 binds to **CTS-N** by pull down (View Interaction)

1. Introduction

ABC transporters couple ATP hydrolysis to the trans-membrane movement of a wide range of substrates. All ABC transporters comprise four functional domains: two sets of primarily α-helical transmembrane domains (TMDs) which associate to form the transport pathway for the substrate and two nucleotide binding domains (NBDs) which dimerise and form two composite ATP binding sites at the dimer interface.

Peroxisomes from all eukaryotes contain at least one member of the ABC subfamily D which transports lipophilic/amphipathic substrates from the cytosol into the peroxisome for β-oxidation. Comatose (CTS; AtABCD1, also known as AtPXA1 and PED3) is a full size peroxisomal ABC transporter from Arabidopsis with the domain organisation [TMD-NBD-TMD-NBD] [1–3]. Thus CTS can be considered to be a fused heterodimer consisting of two homologous but distinct halves. This gene structure is found in all land plants including bryophytes but is not present in algae, suggesting that this fusion happened once in the land plant lineage more than 450 Ma ago (Fig. 1). In contrast, animal and fungal peroxisomal ABC transporters are all half transporters with the organisation [TMD-NBD] that have to dimerise for activity [4]. Mammalian ABCD proteins are functional as homodimers, but in *Saccharomyces cerevisiae*, the half transporters Pxa1p and Pxa2p heterodimerise to transport long chain acyl CoAs [5,6]. We have shown recently that CTS targets to peroxisomes when expressed in oleate-grown *S. cerevisiae* and has the ability to complement the yeast *pxa1 pxa2Δ* mutant for β-oxidation of fatty acids [7].

Since only land plants have peroxisomal transporters with the fused heterodimer configuration, but this arrangement is functional in *S. cerevisiae*, we investigated the targeting and functionality of the

Abbreviations: ABC, ATP Binding Cassette; TMD, transmembrane domain; NBD, nucleotide binding domain

* Corresponding author.
E-mail address: freddie.theodoulou@rothamsted.ac.uk (F.L. Theodoulou).

[1] These authors contributed equally to this work.
[2] Current address: Faculty of Life Sciences, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK.
[3] Current address: Biology Department, Brookhaven National Laboratory, Upton, NY 11973-5000, USA.

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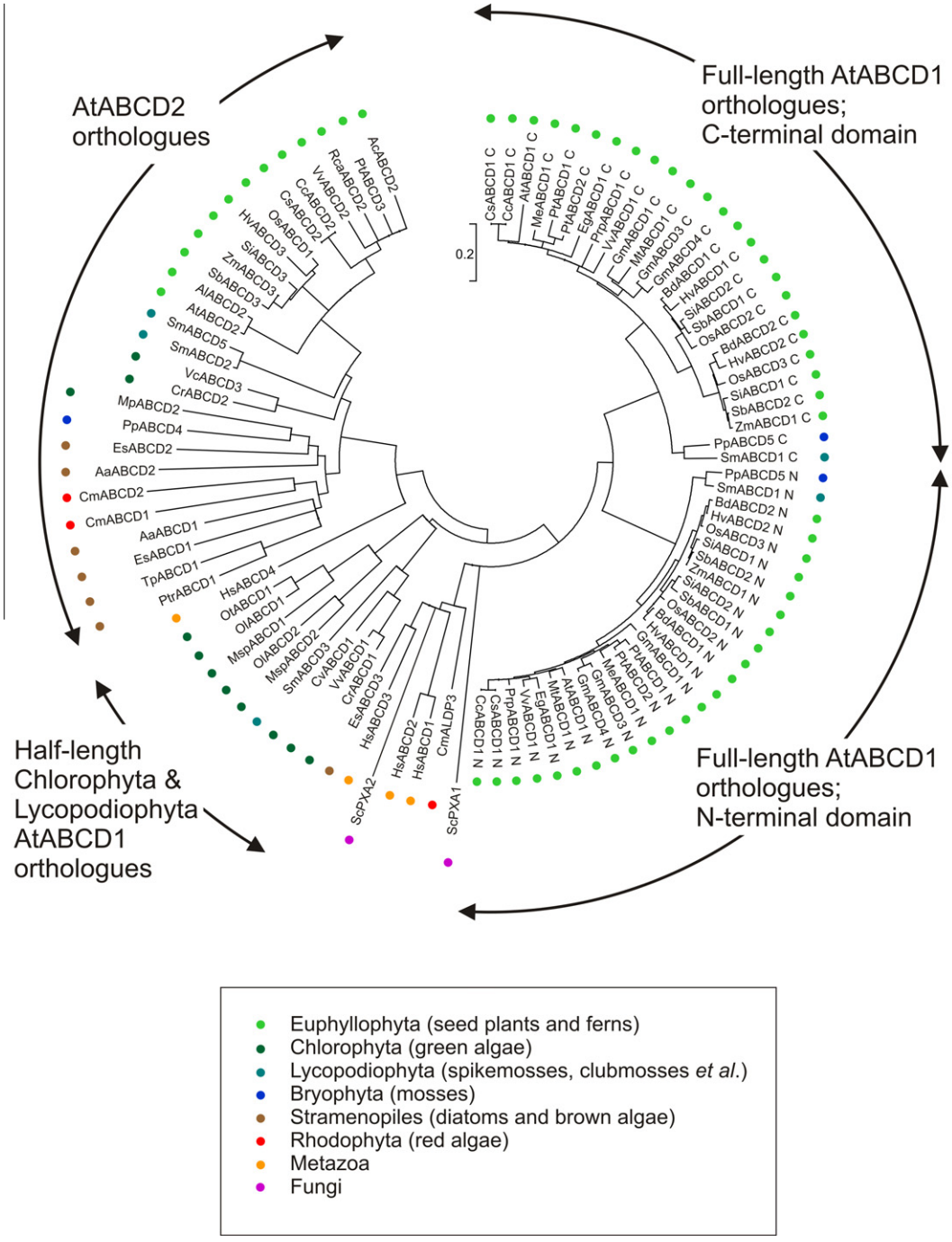

Fig. 1. Phylogenetic analysis of ABC family D transporters. Analysis by the Maximum Likelihood method was performed as described in Supplementary data. Upper and lower case letters at the beginning of protein names indicate abbreviated Latin binomial names for species, as detailed in the species key (*Table* S2). Subfamily members within each species are classified according to [18].

two CTS pseudo half transporters CTS-N and CTS-C from Arabidopsis when expressed in yeast.

2. Materials and methods

2.1. Growth of yeast

Yeast strains were grown as described in [7]. Yeast strains are given in Table 1.

2.2. Pull-down assays

AtPEX19-1 was amplified with primer pair FT119/FT120 (Table S1), restricted with *Bam* HI/*Not* I and cloned in the corresponding sites of pGEX-4T-3 (GE Healthcare). AtPEX19-2 was amplified with primer pair FT121/FT122, restricted with *Sal* I/*Not* I and cloned in the corresponding sites of pGEX-4T-3. Expression and purification of glutathione S-transferase (GST) fusion proteins was carried out as described in [8]. CTS-N and CTS-C were amplified from plasmid H1A6T7 using primer pairs FT187/FT189 and FT190/FT188 respec-