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Minireview

Peroxisomal ABC transporters

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Abstract **Peroxisomes perform a range of different functions, dependent upon organism, tissue type, developmental stage or environmental conditions, many of which are connected with lipid metabolism. This review summarises recent research on ATP binding cassette (ABC) transporters of the peroxisomal membrane (ABC subfamily D) and their roles in plants, fungi and animals. Analysis of mutants has revealed that peroxisomal ABC transporters play key roles in specific metabolic and developmental functions in different organisms. A common function is import of substrates for β-oxidation but much remains to be determined concerning transport substrates and mechanisms which appear to differ significantly between phyla.**

Keywords: Peroxisome; ABC transporter; β-Oxidation; ALDP; PXA1; CTS

1. Introduction: peroxisome functions and the requirement for import of substrates

Peroxisomes are (virtually) ubiquitous organelles of eukaryotic cells which exhibit marked morphological and metabolic plasticity, depending on organism, cell type and prevailing environmental conditions. They perform a range of functions in different taxa, some of which are organism specific and some of which are common to all eukaryotes (Table 1; [1]). Although this diversity of function is reflected in the plasticity of peroxisomes, β-oxidation of fatty acids and the generation and degradation of hydrogen peroxide are the distinctive general biochemical characteristics of this organelle [2,3].

The peroxisome is bounded by a single membrane of similar composition to the endoplasmic reticulum. In order to carry out its various metabolic and developmental functions, the peroxisomal membrane must regulate import and export of metabolites and proteins and plays an important role in co-ordination of peroxisomal metabolism with that of other compartments, in particular, the mitochondrion and also plastids in photosynthetic organisms. Although major advances have been made in understanding the protein import apparatus of peroxisomes [4], the roles of integral and membrane-associated proteins in transport of solutes are relatively poorly characterised, except in yeast where molecular genetic approaches have led to considerable advances in understanding peroxisomal function [5,6]. This review focuses on the roles of ATP binding cassette (ABC) superfamily transporters in peroxisome biology.

2. Properties of the peroxisomal membrane

The classical concept of a biological membrane is of a lipid barrier that is selectively permeable to solutes and macromolecules owing to the presence of specific proteins which mediate transport of these entities. Whilst hydrophobic and apolar molecules can cross the lipid bilayer by diffusion, polar, hydrophilic solutes and large molecules require specific proteinaceous transport systems. In the case of the peroxisome, a number of authors have suggested that the peroxisomal membrane is essentially porous to a range of small molecules [7–10]. This view is supported by studies with osmotically shocked peroxisomes in which membranes were damaged yet the matrix retained its metabolic integrity. This was ascribed to arrangement of matrix enzymes into an aggregated core of multienzyme complexes which functions as a metabolon [8,10–12]. Several studies have described relatively non-specific porins, thought to be responsible for transfer of metabolites across the peroxisomal membrane [10,12–14]. However, the latter scenario is inconsistent with more recent findings: although there are conflicting reports concerning peroxisomal pH values, several groups have demonstrated the existence of a pH gradient between the cytosol and peroxisomal matrix, suggesting that transport of protons between these compartments is controlled [15–17]. Whilst the membranes of isolated peroxisomes appear to be porous to small substrates, the transport of bulky cofactors such as NAD(P)H and CoA is indeed restricted [18] and genetic manipulation of a number of peroxisomal transport systems has confirmed the view that import and export of a range of ions, cofactors, metabolites and proteins is tightly controlled and protein-mediated in vivo as is the case for other organelles [15–17,19–22].

3. β-Oxidation and fatty acid transport

β-Oxidation is the major process for the degradation of fatty acids and also the phylum-specific biosynthesis of important

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Table 1
Metabolic functions associated with peroxisomes

Metabolic pathway	Function	Species distribution	Consequence of mutation	References
Core β-oxidation of fatty acids	Energy production; membrane lipid turnover; membrane breakdown in senescence	Essentially all	Inability to grow on fatty acids as sole carbon source (fungi); reduced pathogenicity (fungi). Defects in germination, seedling establishment or reproductive development depending on the specific defect (plants) Neurological abnormalities, developmental delay, hypotonia, cranio-facial abnormalities and seizure depending upon the specific defect (mammals)	[23,24,131,132]
β-Oxidation of aromatic and cyclic compounds	Production of signal molecules from precursors. For example, production of JA requires specific peroxisomal acyl CoA synthetases and OPR3 plus core β-oxidation pathway. Synthesis of IAA from IBA and possibly salicylic acid from *trans*-cinnamic acid, likely requires specialised activating enzymes and thioesterases	Arabidopsis, tomato, probably all plants	Complete block in JA biosynthesis results in sterility due to failure of pollen production and compromised defence responses Mutants in conversion of IBA to IAA compromise β-oxidation, peroxisome biogenesis and also responses to endogenous IBA affecting root elongation and lateral root initiation	[115,133–137]
	Prostaglandins and leukotrienes are also (partly) degraded by β-oxidation in peroxisomes in mammalian cells	Mammals		[138,139]
α-Oxidation of fatty acids	Breakdown of phytanic acid and other β-methyl fatty acids (mammals). Possible (unproven) role in chlorophyll breakdown (plants). Possible role in response to pathogens (plants)	Mammals, plants	Defects in phytanoyl CoA hydroxylase in humans results in Refsum disease	[24,131,140]
Branched chain fatty acid oxidation	Breakdown of branched chain fatty acids such as pristanoyl-CoA, di and trihydroxycholestanoyl CoA (derived from cholesterol) leading to the production of bile salts	Mammals	2-Methylacyl-CoA racemase deficiency results in adult onset sensory motor neuropathy	[131,141]
Branched chain amino acid catabolism	Breakdown of valine. β-Hydroxyisobutyryl CoA hydrolase is peroxisomal in Arabidopsis. A number of related proteins with putative peroxisome targeting signals are present in the Arabidopsis genome. Breakdown of leucine: HMG-CoA lyase may be targeted to both peroxisomes and mitochondria in Arabidopsis and mammals	Plants, mammals?	Accumulation of toxic methylacrylyl-CoA inhibits β-ketoacyl thiolase and blocks β-oxidation, leading to a β-oxidation deficiency	[137,142–144]
Glyoxylate cycle	Conversion of fat to carbohydrate (gluconeogenesis)	Plants, fungi, including *Leptosphaeria maculans*, and some yeasts	Compromised seedling establishment under sub-optimal conditions (Arabidopsis *icl* mutant); reduced pathogenicity (*Leptosphaeria maculans*; *Candida albicans icl* mutants)	[145–148]

Table 1 (*continued*)

Metabolic pathway	Function	Species distribution	Consequence of mutation	References
	Mammals lack the glyoxylate cycle but glyoxylate can be converted to glycine in the liver by alanine glyoxylate transferase (AGT) which is peroxisomal in humans and many other vertebrates		Patients with defective AGT convert glyoxylate to oxalate resulting in the disorder primary hyperoxaluria type I	
Plasmalogen biosynthesis	Synthesis of ether-linked phospholipids (plasmalogens) which are particularly important in membranes of neural cells. Dihydroxyacetone phosphate acyl transferase (DHAPAT) or alkyldihydroxyacetonephosphate synthase (ADHAPS) which catalyse the first 2 steps of the pathway, are peroxisomal in humans and trypanosomes	Mammals, trypanosomes	All patients with mutations in DHAPAT or ADHAPS show signs and symptoms of rhizomelic chondrodysplasia punctata which include short stature, facial abnormalities, spasticity, severe mental retardation and abnormal bone ossification	[131,149,150]
Isoprenoid biosynthesis	Synthesis of sterols such as cholesterol and quinones such as ubiquinone. HMG-CoA reductase is peroxisomal in trypanosomes. Conflicting data concerning the peroxisomal location of isoprenoid synthesis in mammals	Mammals, trypanosomes		[149,151,152]
Glycolysis	Compartmentalisation of glycolysis within specialised peroxisomes called glycosomes allows glycolysis to continue anaerobically whist maintaining the redox balance as well as compensating for the lack of regulation of trypanosome hexokinase and phosphofructokinase	Trypanosomes	Loss of compartmentation of glycolytic enzymes is highly detrimental. Blood stream form trypanosomes die when peroxisome biogenesis is inhibited or cytosolic phosphoglycerate kinase is expressed	[149,153]
Pentose phosphate pathway	Recycling of $NADP^+$ via glucose–phosphate dehydrogenase and 6-phosphogluconate dehydrogenase	Plants, trypanosomes		[149]
Photorespiration	Recycling of phosphoglycolate produced by oxygenase activity of Ribulose bisphosphate carboxylase oxygenase	Plants	Mutant plants are sensitive to atmospheric levels of oxygen and require elevated CO_2	[154]
Production of glycine-betaine	Betaine aldehyde dehydrogenase (BADH) catalyses the last step of the biosynthesis of the compatible osmolyte glycine-betaine and is peroxisomal in rice and barley	Some plant species	Overproduction of barley BADH in rice increases salt, heat and cold tolerance	[155,156]
Lysine biosynthesis	Biosynthesis of lysine from acetyl CoA and 2-oxoglutarate. LYS4 and LYS1 catalysing the 4th and 11th steps of the pathway have been determined experimentally to be in peroxisomes. LYS9, LYS12 and LYS2 have a putative PTS	*Saccharomyces cerevisiae*	Mutants in the pathway are all lysine auxotrophs. Some mutants are also impaired in growth on lysine deficient media	[157,158]
Penicillin biosynthesis	Acyl-CoA:6-aminopenicillanic acid acetyl transferase which catalyses the final step in penicillin biosynthesis by replacing the α-adipyl side chain with phenylacetate to give penicillin G is peroxisomal. Phenylactetate CoA ligase (PCL) also has a PTS1	*Penicillium chrysogenum*; *Aspergillus nidulans*	*Aspergillus nidulans* mutant defective in peroxisome biogenesis still made penicillin but mistargeting of the acyl transferase abolished penicillin biosynthesis suggesting may need 2 or more enzymes (AT and PCL?) in same compartment	[159,160]

(*continued on next page*)

Table 1 (*continued*)

Metabolic pathway	Function	Species distribution	Consequence of mutation	References
Degradation of purines/ synthesis of ureides	Xanthine is converted to urate by xanthine dehydrogenase which is peroxisomal in some species. Urate oxidase (uricase) and allantoinase are found in peroxisomes from many species. Allantoic acid can be further metabolised to glyoxylate and urea in many fungi. The ureides allantoin and allantoic acid are the major form of fixed nitrogen transported by tropical legumes	Mammals, plants amphibia, fungi (yeasts but not *Saccharomyces cerevisiae*)	Antisense down regulation of uricase resulted in peroxisomes of reduced size and nitrogen deficiency	[161–163]
Catabolism of polyamines	Polyamine oxidases have been identified in plants and mammals that carry putative peroxisome targeting signals	Plants, mammals		[137,164]
Degradation of D-amino acids	D-Amino acids can utilised by many fungi as a carbon and nitrogen source via the peroxisomal enzyme D-amino acid oxidase	Fungi (yeasts but not *Saccharomyces cerevisiae*)	Inability to grow on D-amino acids	[165]
Methanol metabolism	Many fungi can metabolise methanol via peroxisomal alcohol oxidase and dihydroxyacetone synthase	Fungi (yeasts but not *Saccharomyces cerevisiae*)	Inability to grow on methanol	[165]
Purine salvage pathway	Trypanosomes cannot make purines de novo. Glycosomes contain a hypoxanthine-guanine phosphoribosyl transferase and xanthine phosphoribosyl transferase which convert the bases to the corresponding purine mononucleotide	Trypanosomes		[149]
Pyrimidine biosynthesis	Orotate phosphoribosyl transferase and orotidylate decarboxylase in glycosomes convert orotate to UMP	Trypanosomes		[149]
Active oxygen metabolism	Removal of active oxygen species produced by peroxisomal metabolism	Essentially all		[166,167]

Metabolic processes that are at least partially compartmentalised within peroxisomes in different organisms are listed. Many of these pathways are shared with other cellular compartments, highlighting the need for extensive traffic of metabolites into and out of peroxisomes. For more detail the reader is referred to the cited literature.

lipid-derived molecules such as plasmalogens, bile acids and jasmonates in eukaryotes (Table 1). Most of the genes involved have been identified in animals, yeast and plants [23–25]. In yeast and plants, β-oxidation of straight chain fatty acids is confined to the peroxisome, whereas in mammals, initial chain shortening of very long-chain fatty acids (VLCFA; C $\geqslant$ 22:0) occurs in the peroxisome and β-oxidation is completed in the mitochondrion [23,24,26,27]. Transport of substrates for β-oxidation across the peroxisomal membrane is a key step in this pathway. Prior to β-oxidation, fatty acids are activated by thioesterification to Coenzyme A (CoA), a process which occurs in the cytosol, on the ER or in the peroxisome, dependent on chain length and organism. This determines the nature of the molecular species that must cross the peroxisomal membrane and has implications for the mechanism of transport. Fatty acyl CoAs are amphipathic in nature and therefore require a transport protein to cross lipid bilayers. In contrast, physicochemical properties predict that many fatty acids should be membrane permeable and do not require a transporter [28,29]. However, it has been shown that other membrane-permeable molecules such as ammonium and water have specific membrane-bound transporters and that this permits higher rates of transport, selectivity and co-ordination with other cellular processes. Moreover, there is evidence that fatty acid transport is a saturable, protein-dependent process [30]. Both passive and membrane-protein-mediated transport of free fatty acids can be coupled with activation on one side of the membrane, a process known as vectorial acylation. Thus transport

and activation are intimately linked processes. A number of ABC proteins and acyl CoA synthetases have been implicated in lipid transport [23,25,27,31,32]; here, we review evidence for the roles of peroxisomal ABC transporters in the metabolism of lipids and formation of bioactive lipid metabolites.

4. Peroxisomal ABC transporters

ABC proteins are encoded by the largest gene superfamily in all sequenced genomes to date and compromise a range of mostly membrane-bound proteins with diverse functions. Many of these proteins are involved in transport of a wide variety of molecules (see reviews elsewhere in this volume). ABC transporters are characterised by the sequence and organisation of an ATP binding domain. This domain, also known as the nucleotide binding fold (NBF) contains two conserved motifs: Walker A and Walker B, separated by ca. 90–120 amino acids. A third, so-called ABC signature motif is situated upstream of the Walker B sequence [33]. A functional ABC protein typically comprises two NBFs and two transmembrane domains (TMDs), the latter of which contains several α-helices. In eukaryotic ABC transporters, these domains are generally organised as "full-size" transporters, with the topology TMD–NBF–TMD–NBF (or the reverse: NBF–TMD–NBF–TMD) or as two "half-size" transporters TMD–NBF (or NBF–TMD).

In recent years, characterisation of prokaryotic and eukaryotic genome sequences has allowed the complete identification of ABC genes in genomes representative of all major phyla. [34–41]. The ABC family is divided into subgroups based on phylogenetic relationships between their nucleotide binding domains and members of each subfamily generally exhibit the same domain structure [36]. Peroxisomal ABC transporters belong to subfamily D which is present in all eukaryotic taxa and comprises from one to five members (Table 2). With the exception of plant representatives, all ABCD proteins are "half-size" transporters with the topology TMD–NBF [36]; Table 2. As is the case for other half-size ABCs [42], ABCD proteins dimerise to form functional transporters (Fig. 1). Table 2 lists ABCD subfamily members from completed genomes.

4.1. Mammalian ABCD subfamily

The ABCD subfamily in humans comprises four members, all of which are half-size ABC proteins: Adrenoleukodystrophy protein (ALDP; ABCD1), Adrenoleukodystrophy-related protein (ALDRP; ABCD2) and the 69 and 70 kDa peroxisomal membrane proteins, PMP69 (ABCD4) and PMP70 (ABCD3) [43–48]. Members of the mouse ABCD subfamily are strictly orthologous to those of humans [49] and consequently, use of targeted gene deletions in mouse has proved a useful tool for analysing the functions of mammalian peroxisomal ABC transporters. Whilst ALDP has been extensively studied, and, together with ALDRP and PMP70, has been implicated in β-oxidation of fatty acids (see below), PMP69 has not been well characterised but is known to be ubiquitously expressed [46,48]. The function of PMP69 has not yet been determined, but based on sequence similarity, it has been suggested to have a similar function to PMP70.

4.1.1. ABCD3: PMP70. The 70 kDa peroxisomal membrane protein, PMP70, was the first peroxisomal member of the ABC superfamily to be identified. Discovered as a major integral membrane protein of peroxisomes, it was originally proposed to be a transporter, based on its membership of the ABC superfamily and the cytosolic orientation of the ATP binding domain [43]. Although PMP70 has been shown to restore or partially restore peroxisomal biogenesis in mutant cell lines defective in *PEX2*, an essential component of the peroxisome protein import machinery, the biochemical basis for this is not clear [50,51]. Rather, a role for PMP70 in β-oxidation appears likely: the induction of *PMP70* not only parallels peroxisome proliferation but also the induction of β-oxidation enzymes ([52], and references therein). Strong evidence for a transport function was provided by transfection studies with Chinese hamster ovary (CHO) cell lines: overexpression of rat *PMP70* increased β-oxidation of palmitic acid (C16:0) but not lignoceric acid (C24:0) and did not alter the number or size of peroxisomes, arguing against a role in biogenesis [52]. Latency experiments suggested that PMP70 mediates transport of long-chain fatty acid (LCFA)-CoA across the peroxisomal membrane [52] and mutagenesis of conserved motifs indicated a requirement for ATP binding and hydrolysis [52]. Interactions with 8-azido-[α-^{32}P] ATP consistent with transport function were subsequently demonstrated using both recombinant NBF fusion protein and intact, native PMP70 [53,54] and limited proteolysis revealed conformational changes during the cycle of ATP binding and hydrolysis [55]. In addition to a role in peroxisomal import of straight chain fatty acyl-CoA, PMP70 has recently been suggested to be involved in the transport of 2-methylacyl-CoA esters, including pristanoyl-CoA, and di- and trihydroxychoestanoyl-CoA [56]. Pristanic acid and di- and trihydroxycholestanoic acids are strictly oxidised in peroxisomes with pristanic acid undergoing three cycles of β-oxidation and di- and trihydroxycholestanoic acid only a single β-oxidation cycle in peroxisomes to produce chenodeoxycholic acid and cholic acid, respectively [57].

4.1.2. ABCD1: ALDP. X-linked Adrenoleukodystrophy (X-ALD) is the most common peroxisomal neurodegenerative disease with a frequency of 1 in 21 000 males in the USA [58]. It exhibits a range of clinical phenotypes [59], characterised biochemically by the accumulation of saturated, straight chain, very long-chain fatty acids (VLCFA; C $\geqslant$ 22:0) in plasma and tissue. Since the primary cause of elevated VLCFAs is a defect in β-oxidation, specifically, reduced activity of VLCFA-acyl CoA synthetase (VLACS), it was originally thought that VLACS was the candidate gene for *X-ALD* [60–63]. However, positional cloning identified an ABC transporter with similarity to a 70 Da peroxisomal protein as the *X-ALD* gene [44] and mutations in the *X-ALD* gene have been found in all X-ALD patients to date [64]. The gene product, designated ALDP, was subsequently shown to be an integral peroxisomal membrane protein with a cytosolic ATP binding domain [65–68]. Expression of ALDP corrects β-oxidation of VLCFA in X-ALD fibroblasts, supporting the notion that ALDP and not VLACS is responsible for X-ALD [69–71]. A wide variety of mutations has been associated with X-ALD, including intragenic deletions, point mutations, micro deletions and insertion events [64,72,73]. Around 50% of mutations described to date are missense mutations which predominantly affect specific, functionally important domains: regions within the TMD which may be concerned with protein: protein interactions and peroxisomal targeting, a highly

Table 2
ABCD proteins from genome-sequenced organisms

Organism	Name	Synonyms	Accession No.	Domain organisation	References
Homo sapiens	ABCD1	ALDP	P33897	TMD–NBF	[36,44]
	ABCD2	ALDR, ALDL1,	Q9UBJ2		
	ABCD3	PMP70, PXMP1	P28288		
	ABCD4	PMP69, P70R, PXMP1L	O14678		
Mus musculus	ABCD1	ALDP	P48410	TMD–NBF	[49]
	ABCD2	ALDR	Q8BQ63		
	ABCD3	PMP70	Q91VT3		
	ABCD4	PMP69	O89016		
Caenorhabditis elegans	PMP1	C44B7.8	Q 18597	TMD–NBF	[41,102]
	PMP2	C44B7.9	Q18598		
	PMP3	C54G10.3	Q 18843		
	PMP4	T02D1.5	O45730		
	PMP5	T10H9.5	O76414		
Drosophila melanogaster		DmCG12703	Q9VWC8	TMD–NBF	[36,39]
		DmCG2316	Q8IMC0		
Anopheles gambae			Q7QFE4	TMD–NBF	[39]
			Q7PYR2		
Arabidopsis thaliana	CTS	PXA1/PED3/AtPMP2	Q94FB9	TMD–NBF–TMD–NBF	[111–113]
Oryza sativa	OsCTSl		Q84ZW6	TMD–NBF–TMD–NBF	[40]
	OsCTS2		Q5JJV5		
Saccharomyces cerevisiae	PXA1	PAT2/PALl/YPL147w	P41909	TMD–NBF	[34,106]
	PXA2	PAT1/YKL188c	P34230		
Aspergillus fumigatus			Q8TFX6	TMD–NBF	
			Q4WJU3		
Candida albicans			Q5A3L5	TMD–NBF	
			Q5AAI9		
Candida glabrata			Q6FJZ4	TMD–NBF	
			Q6FWG6		
Neurospora crassa			Q9P6C3	TMD–NBF	
			Q7SD69		
Cryptococcus neoformis			Q5KK89	TMD–NBF	
			Q55W02		
			Q5KNJ1		
			Q55Z76		
Dictyostelium discoideum	ABCD.1		Q8T8P5	TMD–NBF	[38]
	ABCD.2		Q8T8P3		
	ABCD.3		Q8T8P4		

ABCD genes and proteins were identified from the literature surveys (indicated by references) and by BLAST searching non-redundant protein databases using ALDP, CTS and PXA1,2 as input sequences.

conserved "EAA" motif and the NBF [64]. Many mutations result in protein instability or absence, such that up to 70% of ALD patients lack immunoreactive ALD [67]. An up-to-date survey of ALD mutations can be found at http://www.x-ald.nl. Confusingly, there is no clear cut association with genotype and phenotype in X-ALD patients, for example, no correlations can be made between nature of the mutation, level of VLCFAs and severity of pathology [64,72]. The intra-familial variability of ALD symptoms has so far defied any attempt at a simplistic biochemical explanation and has led to the suggestion that modifier genes may be responsible for variable expression of the disease. The medical importance of X-ALD and the ethical issues with human research prompted the development of a mouse model. Targeted deletion of ALDP by three groups replicated the biochemical defect of X-ALD, but knockout mice did not exhibit severe neurological symptoms until very late in development, further underlining the clinical complexity of X-ALD [74–76].

4.1.3. Function of ALDP and relationship with VLACS. Currently, the role(s) of ALDP in VLCFA metabolism and the pathology of X-ALD are unclear and the biochemical function of ALDP has yet to be determined unequivocally. It has been variously suggested that ALDP is directly involved in β-oxidation of VLCFA through transport of VLCFAs, VLCFA-CoA, or the cofactors CoA, or ATP. Substrate-induced conformational alterations suggest that

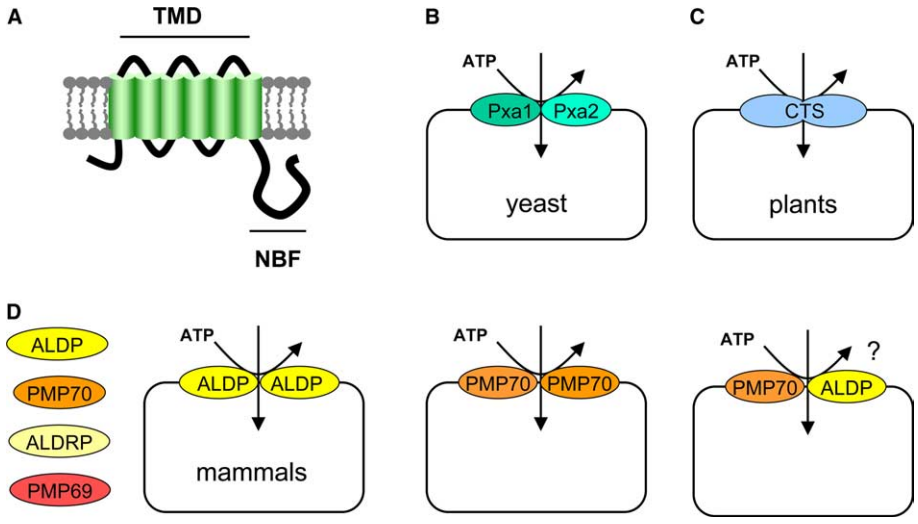

Fig. 1. Domain organisation of the ABCD subfamily. (A) Cartoon of a generalised "half-size" ABC transporter. In ABC subfamily D, the TMD precedes the cytosolically oriented NBF. The number of α-helices in the TMD of ABCD proteins has not been established experimentally and may vary between different proteins. (B–D) ABCD proteins in peroxisomes of yeast, plants and mammals. Yeast has two half-size ABCD proteins, Pxa1p and Pxa2p, which form a heterodimer in vivo and in vitro (B), whereas plants have a single, full-size peroxisomal ABC protein, CTS, which is essentially a fused heterodimer (C). Mammals contain four different half-size proteins: ALDP, ALDRP and the 69 and 70 kDa peroxisomal proteins, PMP69 and PMP70. Although these proteins can heterodimerise in vitro, it is still not unequivocally established whether this happens routinely in vivo (D).

ALDP is directly involved in transport of L- and VLCFA-CoAs across the peroxisomal membrane [77]. Alternatively, roles in transport or stabilisation of the VLACS protein have been proposed [44,64,65] and overexpression and knockout studies of *ALDP* and *VLACS* have sought to test these hypotheses. VLCFA accumulate in tissues of *ALDP* deficient X-ALD mice and fibroblasts from these animals show reduced rates of VLCFA β-oxidation, similar to findings for X-ALD patients [74–76]. Immunoreactive VLACS was present in the peroxisomal matrix of human and mouse X-ALD fibroblasts, indicating that X-ALD is required neither for transport nor stabilisation of VLACS [78–80] but co-expression of *ALDP* and *VLACS* had a synergistic effect on both β-oxidation and VLACS activity, suggesting a functional, rather than a physical interaction [79]. It has been assumed that reduced degradation evident in studies of fibroblasts was responsible for VLCFA accumulation in X-ALD tissues, however, it was demonstrated recently that peroxisomal β-oxidation of VLCFA is normal in *tissues* of X-ALD mice [81]. Furthermore, expression of *VLACS* in X-ALD cells improved β-oxidation [82], contradicting an earlier report [79] and mice deficient in peroxisomal VLACS had reduced peroxisomal β-oxidation but normal VLCFA levels [83]. Thus peroxisomal β-oxidation and the accumulation of VLCFA are uncoupled in the mouse model: ALDP is not necessary to maintain VLACS activity and the control of VLCFA levels does not depend on the direct interaction of VLACS and ALDP.

This apparent paradox has led to the hypothesis that an indirect mechanism involving mitochondrial dysfunction might be responsible for the accumulation of VLCFA in X-ALD [81]. A close relationship between mitochondrial and peroxisomal β-oxidation was established and inhibition of mitochondrial β-oxidation was accompanied by a significant reduction of peroxisomal VLCFA β-oxidation. It was proposed that *ALDP* deficiency influences mitochondrial function causing an increase in LCFA. Since peroxisomal VLACS has a higher affinity for LCFA than for VLCFA, the net consequence would be saturation of peroxisomal VLACS, resulting in VLCFA accumulation. It was further suggested that accumulation of VLCFA could cause mitochondrial damage [81]. However, a detailed study of mitochondrial ultrastructure and function in X-ALD tissues indicated that this was not the case and also that mitochondrial dysfunction did not underpin accumulation of VLCFA [84]. This raises the question: what is the cause of elevated VLCFA in X-ALD cells, if not impaired degradation? The answer may lie in VLCFA synthesis: X-ALD cells exhibit increased fatty acid elongation, which could contribute to increased levels of VLCF As in X-ALD [85]. However, it is not known how mutations in ALDP relate to microsomal fatty acid elongation.

4.1.4. ABCD2: ALDRP. ALDRP is highly similar to ALDP (88% similarity and 67% identity at the amino acid level) [45,47] and both gene structure and phylogenetic analysis suggest relatively recent divergence from a common ancestor [86]. The expression patterns of *ALDP* and *ALDRP* are distinct but overlapping with predominant expression of *ALDRP* in brain and heart [45,47,87–89]. This, together with close sequence homology, has led to the suggestion that ALDRP and ALDP are functionally redundant, performing a similar role in different cell types. Strong evidence in support of this hypothesis has recently been obtained ([88,89] and see below).

4.1.5. Rescue of X-ALD: functional implications and role of ALDRP. X-ALD fibroblasts have approximately 30% of wild type VLCFA β-oxidation activity, therefore absence of ALDP results in the reduction but not elimination of VLCFA β-oxidation, suggesting either that there are compensatory or redundant activities or that the effect of ALDP on VLCFA β-oxidation is indirect. X-ALD fibroblasts also have normal levels of LCFA (C16:0) oxidation, which is consistent with the endogenous expression of *PMP70* in this cell type [61,62,74]. Expression not only of *ALDP* and *ALDRP* but also of *PMP70* restores VLCFA β-oxidation in X-ALD fibroblasts

[70,90–92] indicating a degree of functional overlap. The latter observation is surprising given that PMP70 is implicated in transport of LCFA-CoA and overexpression in CHO cells did not increase VLCFA β-oxidation [52] and perhaps indicates a non-specific effect. Peroxisomal VLCFA β-oxidation could also be restored by treatment of X-ALD cells or animals with the peroxisome proliferator, fenofibrate [92] and with pharmacological agents such as sodium 4-phenylbutryate (4PBA) [90]. Initially, correction of VLCFA β-oxidation and VLCFA levels was attributed to increases in *ALDRP* expression and pharmacological induction of functionally redundant transporters was proposed as a promising therapeutic approach for X-ALD [88,90,92]. Although some reports suggest that enhancement of VLCFA β-oxidation by 4PBA and other pharmacological agents is associated with induction of mitochondrial LCFA β-oxidation, rather than with induction of *ALDRP* [81,93], the neurological and biochemical phenotypes of X-ALD mice can be ameliorated by overexpression of *ALDRP* [88]. Moreover, double ALD^{-},$ALDR^{-}$ mutants exhibited a more severe phenotype than *ALD*-single mutants. This provides unequivocal evidence that ALDP and ALDRP share at least some functionality but also suggests that ALDRP has additional roles in vivo. More detailed analysis revealed that *ALDRP* expression in neuronal tissue is highly cell-type specific: although deletion of *ALDRP* did not lead to higher levels of VLCFA in neural tissue in general [88], VLCFA were indeed elevated in dorsal root ganglia, a cell population in which *ALDRP* expression is high [89]. Levels of other peroxisomal metabolites were normal. Interestingly, the electrophysiological and neurological phenotypes of *ALDP*- and *ALDRP*-mutant mice were distinct, perhaps reflecting the roles of ALDP and ALDRP in different cell types. It could not be concluded whether elevated VLCFA was a direct or indirect effect of deletion of either gene. Whilst the precise biochemical function(s) of ALDP and ALDRP remain unclear, the genetic evidence from analysis of X-ALD patients and knockout mice nevertheless points to important roles in normal physiology.

4.1.6. Interactions and function: hetero and homodimerisation. Since humans have four peroxisomal ABC transporters [43–46], it has been suggested that these peroxisomal half-transporters dimerise in the peroxisomal membrane to form functional "full-size" transporters and that heterodimerisation might contribute to functional diversity [94]. Homodimerisation of ALDP and heterodimerisation of ALDP with ALDR and PMP70 have been demonstrated in vitro using yeast two-hybrid assays and co-immunoprecipitation of recombinant proteins [64,95]. Native complexes of ALDP and PMP70 have also been isolated by coimmunoprecipitation and a tentative link between transporter phosphorylation and complex formation established [54]. Despite the biochemical evidence for heterodimerisation, X-ALD is associated only with mutations in ALDP and not with any of the remaining three peroxisomal ABC transporters. If dimerisation of ALDP were required for function, then X-ALD mutations in a heterodimerisation partner would be expected on statistical grounds. Moreover, the distinct expression patterns of ALDP, ALDR and PMP70 in mice and humans suggest that they function independently as homodimers [87,89,96–98]. Whilst it is clear that heterodimers can be formed in vitro, the stoichiometry of ABC transporter expression must be an important factor in determining interactions between the different half-transporters and recent protein purification studies employing stringent detergent solubilisation conditions suggest that homomeric interactions of ALDP and PMP70 prevail in vivo [99]. Altering the expression of one or more protein could impact on peroxisomal function in two ways: by competing for membrane insertion sites with endogenous transporters or by forming heterodimers with altered or compromised function. Interestingly, overexpression of PMP70 in CHO cells promotes β-oxidation of LCFA suggesting that it acts as a homodimer in vivo. However, β-oxidation of VLCFA was depressed by 30–40% in the same cells, suggesting displacement of ALDP or heterodimerisation with PMP70 [52]. Similarly, co-expression of normal and mutated ALDP reduced protein function, suggesting either that peroxisomes contain a limited number of sites for integration of ALDP or that ALDP homodimers were titrated out by the formation of non-functional mutant/wild type heterodimers [100].

In addition to forming homo- and heterodimers, peroxisomal ABC transporters are expected to be intimately associated with other peroxisomal membrane proteins, for example, yeast two-hybrid and GST pull-down assays have shown that PEX19 interacts with ALDP, ALDRP and PMP70, and is likely to be involved in trafficking these proteins to the peroxisomal membrane [101].

4.2. Nematode ABCD subfamily

The model nematode, *Caenorhabditis elegans*, contains five putative ABCD transporters, two of which are closely linked and may be subject to common regulation [41,102] (Table 2). RNAi silencing of genes encoding peroxisomal proteins demonstrated that, although enzymes of β-oxidation are not required for post-embryonic development in *C. elegans*, a defect in *CePMP1*, *2* and *4* results in arrest at the first stage in larval development, indicating the importance of peroxisomal ABC transporters in worm physiology [102].

4.3. Yeast ABCD subfamily

Despite extensive studies of peroxisomal ABC proteins in mammalian systems, the functions of ABCD transporters are perhaps best understood in baker's yeast, *Saccharomyces cerevisiae*. Yeast represents a simple yet powerful experimental system with specific advantages for analysing β-oxidation: firstly, this pathway is restricted exclusively to peroxisomes, secondly, yeast is able to use fatty acids as the sole carbon source and this property, together with the ready availability of disruption mutants permits rapid analysis of gene function. This prompted a search for yeast homologues of *ALDP* and *PMP70* which were initially isolated during the genome sequencing project [103] and in PCR screens for ABC transporter sequences using RNA from oleic acid-induced yeast [104,105]. Yeast contains two *ALDP* homologues: *PXA1* (*pero*xisomal *A*BC-transporter *1*), also known as *PAT2* or *PAL1*, and *PXA2*, also known as *PAT1* [104–106]. Cellular fractionation and immunogold microscopy demonstrated that Pxa1p and Pxa2p are both integral peroxisomal membrane proteins [104–106].

The Δ*pxa1* and Δ*pxa2* deletion mutants are unable to grow on oleate (C18:1) as the sole carbon source and exhibit reduced β-oxidation of this LCFA. However, *PXA1* and *2* are not required for peroxisome biogenesis or for growth on medium chain fatty acids such as laureate (C12:0) or

myristate (C14:0) [104–106], indicating a specific function for these transport proteins in β-oxidation of LCFA. Since the phenotypes of both single mutants and the double mutant are identical, it has been proposed that Pxa1p and Pxa2p operate as a heterodimer to form a functional transporter [106], and this has been demonstrated experimentally by co-immunoprecipitation [107]. Studies using permeabilised yeast spheroplasts established that Pxa2p was required for the peroxisomal transport of the C18:1-CoA, a long-chain acyl CoA ester, but not for import of C8:0-CoA [108], suggesting a model in which LCFA are activated in the cytoplasm and are imported into the peroxisome by the Pxa1p/Pxa2p heterodimer (Fig. 2; [106,108]). In contrast, medium chain free fatty acids (MCFAs) enter yeast peroxisomes as free acids, and are activated by peroxisomal acyl CoA synthetase, Faa2p [106]. The peroxisomal membrane protein, Pex11p, has been shown to be required for MCFA β-oxidation [109]. Although Pex11p is not an ABC transporter, it is co-regulated with the β-oxidation machinery and latency studies suggest that Pex11p could provide the MCFA-CoA synthetase (Faa2p) with substrate [109]. However, the latter hypothesis has been challenged by analysis of *PEX11* knockout mice [110].

4.4. Plant ABCD subfamily

An inventory of Arabidopsis ABC proteins assigned two genes to the ABCD subfamily, designating them as *AtPMP1* (A1g54350) and *AtPMP2* (At4g39850) (*Arabidopsis thaliana* peroxisomal membrane protein; [37]). Although the protein encoded by *AtPMP1* is a half-size transporter with 31% amino acid identity to ALDP, orthology analysis does not group it with ALDP and examination of the peptide sequence reveals a putative chloroplast transit peptide (http://www.tigr.org/tigr-scripts/tgi/). The second gene, *AtPMP2*, has been identified in three independent forward genetic screens and is also known as *CTS* (*COMATOSE*), *AtPXA1* (*Arabidopsis thaliana peroxisomal ABC transporter 1*) or *PED3* (*peroxisomal defective 3*), referred to hereafter as *CTS* [111–113]. Unlike the yeast and mammalian peroxisomal ABCs, *CTS* encodes a full-size, 1337 aa ABC protein with predicted molecular mass of ca. 150 kDa. This was confirmed experimentally by immunoblotting, and CTS was also shown to be an integral peroxisomal membrane protein [111,112]. The N- and C-terminal halves of the protein are each around 35% identical to ALDP, suggesting that open reading frames encoding two half-size transporters have become fused during the course of evolution to specify a single protein product with the topology

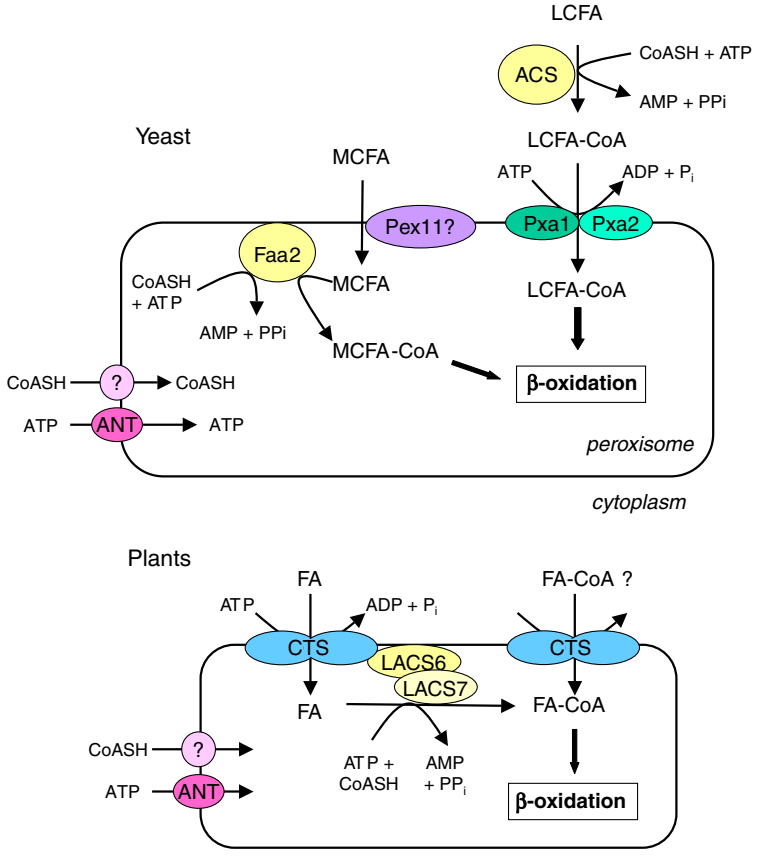

Fig. 2. Peroxisomal fatty acid import in yeast and plants. In yeast, LCFAs are activated in the cytoplasm by ACS. LCFA-CoA esters are imported into the peroxisome by the Pxa1p/Pxa2p heterodimer. In contrast, MCFAs are imported independently of Pxa1p/Pxa2p and activated in the peroxisome by the action of the acyl CoA synthetase Faa2p. Faa2p requires a peroxisomal pool of ATP and CoA which are supplied by the peroxisomal ANT and an as-yet characterised CoA transporter (?) respectively. The peroxin, Pex11p, has been suggested to play a role in the import of MCFA, although it is not thought to be a transport protein per se. In plants, the transport substrate has not been identified experimentally, but it is plausible that free FAs are imported by the action of CTS and esterified to CoA by one of the peroxisomal acyl CoA synthetases (LACS6 and LACS7). However, it cannot be excluded that acyl CoAs are imported, as is the case for yeast. The import of cofactors ATP and CoA is likely to be similar to that in yeast.

TMD–NBF–TMD–NBF. This gene structure appears to be conserved in plants, since examination of the rice genome sequence reveals the presence of two sequences encoding full-size proteins with high identity to ALDP (Table 2; [40]). Two genes also appear to be represented in barley and other grass EST databases, suggesting either that gene duplication has occurred after the monocot/dicot divide, before the evolution of the grasses, or that duplication occurred prior to the divergence of monocots and dicots and the second copy has been lost from the Arabidopsis genome (not shown). Thus, plants can be considered to contain one or more ''fused heterodimers''.

The isolation and characterisation of mutant alleles have provided considerable insight into the functions of peroxisomal ABC transporters in plants. The *ped3* allelic series and the *pxa1-1* mutant were isolated in screens for resistance to the auxin precursors 2,4-dichlorophenoxybutyrate (2,4-DB) and indole butyric acid (IBA), [112–115]. By analogy with β-oxidation of fatty acids, 2,4-DB and IBA are thought to be activated by CoA esterification and undergo one cycle of β-oxidation to shorten the acyl chain by two units, yielding 2,4-dichlorophenoxyacetate (2,4-D) and indole acetic acid (IAA), respectively. The conversion to these bioactive auxins causes stunting of roots in wild type plants (Fig. 3). Although exogenous application of 2,4-D and IAA also inhibits root growth in *ped3* and *pxa1-1*, the mutants are unable to convert 2,4-DB and IBA and therefore can elongate their roots in the presence of these precursor compounds. This implies that the lesion in these mutants is a block in β-oxidation, not only of auxin precursors, but also of fatty acids. In oilseeds such as Arabidopsis, β-oxidation plays an important role in mobilisation of seed storage lipids, which, together with glyoxylate cycle, is essential for the provision of energy and carbon skeletons for seedling establishment. In agreement with this role, *ped3* and *pxa1-1* mutants are unable to establish in the absence of exogenous sucrose. This phenotype is shared with other Arabidopsis β-oxidation mutants [25]. It should be noted that not all seeds store lipid as the major energy source for establishment. Nevertheless, the aleurone layer of starchy seeds such as those of wheat and barley contains oil which plays an important role in starch mobilisation by providing energy for the synthesis of copious quantities of α-amylase [116]. Thus a role for *CTS* orthologues can be envisaged in germination and establishment of cereals as well as in oilseeds.

In apparent contrast to *ped3* and *pxa1-1*, *cts-1* was isolated as a mutant severely compromised in germination [111,117]. Analysis of the *cts-1* locus reveals a chromosomal translocation at a position in the open reading frame corresponding to amino acid 616. A second allele, *cts-2*, isolated by reverse genetics, contains a T-DNA insertion at aa 117, and has a very similar phenotype to *cts-1* [111]. Absence of transcripts and protein from *cts-1* and *cts-2* suggests that they are essentially null alleles ([111]; Baker et al., unpublished data), but the block in germination can be overcome to varying degrees by classical dormancy-breaking treatments such as mechanical rupture of the seed coat and chilling of imbibed seeds prior to assay for germination. In common with *ped3* and *pxa1-1*, *cts-1* and *cts-2* are unable to establish in the absence of sucrose, however, rescued *cts* plants become photosynthetic following establishment and can complete a full life cycle [111]. Although exogenous sucrose permits establishment, it does not rescue the germination defect of *cts*, implying that *CTS* has a distinct function in the transition from dormancy to germination. Since the *ped3* and *pxa1-1* mutants do not appear to be affected in germination [112,113], it was proposed that these represent weaker alleles, in apparent agreement with the position of the respective mutations in the CTS protein [111]. However, comparative germination studies under controlled conditions suggest that the germination phenotype of *pxa1* at least, is almost as severe as that of *cts-1* and *cts-2* (S. Footitt, G. Forbes, J. Marquez, H. Schmuths, E. Carrera, A. Baker, F. Theodoulou and M. Holdsworth, manuscript in preparation). An interesting task for future research will be to elucidate the biochemical role of CTS in the transition from dormancy to germination.

Electron microscopic examination of cotyledons from *cts-1* seedlings revealed a marked inability to break down lipid bodies. Accordingly, profiling of fatty acids and acyl-CoA esters demonstrated that around 70% of storage lipid-derived fatty acids were retained in *cts-1* and *cts-2*. Most notably, levels of fatty acyl CoAs were higher in the mutants, in particular the C20:1, C20:0 and C22:1 VLC-CoAs which are markers for Arabidopsis storage lipid [111]. This phenotype is common to other β-oxidation and associated single and double mutants, including peroxisomal long-chain acyl CoA synthetase (*lacs6 lacs7*), acyl CoA oxidase (*acx1 acx2*), thiolase (*kat2*) and peroxisomal citrate synthase (*csy2 csy3*) (reviewed in [25]). Accumulation of CoAs had no effect on peroxisome morphology in *cts* and *lacs6 lacs7*, but caused enlargement and distortion of peroxisomes in downstream mutants. This is interpreted as reflecting the intracellular location of CoAs: in downstream mutants, CoAs must accumulate in the peroxisome, whereas in *cts* and *lacs6 lacs7*, they are presumed to be extra-peroxisomal, suggesting the existence of an as-yet uncharacterised cytosolic or microsomal acyl CoA synthetase [25,118].

The retention of FA and acyl-CoAs in *cts* mutants and the fact that *CTS* encodes an integral membrane protein suggest that CTS mediates transport of FA, FA-CoA or possibly cofactors for β-oxidation into the peroxisome. Although other scenarios are possible, (for example, CTS might regulate the activity of another transporter or of enzymes involved in lipid catabolism) this remains the simplest hypothesis. If this is the case, what are the substrates for CTS? CTS is unlikely to be an ATP transporter, since a peroxisomal adenine nucleotide translocator (ANT) belonging to the mitochondrial carrier protein family has been identified in yeast and Arabidopsis [21,119]. Similarly, in mammals and yeast, CoA is imported into mitochondria by a carrier protein, rather than by an ATPase, and it is plausible that a similar transporter is present in peroxisomes [120], although some authors suggest that a specific CoA transporter is not required by this organelle [18]. Thus, it is most likely that CTS transports substrates for β-oxidation, although it should be noted that this hypothesis awaits unequivocal experimental support. The retention of acyl CoA in *cts-1* and *cts-2* could be interpreted as evidence that the substrate for CTS is acyl-CoA, as is the case for the yeast heterodimeric transporter, Pxa1p/Pxa2p [108]. However, retention of CoA is also consistent with FA as substrate for CTS: since C20:1 is not a component of structural lipids, it may accumulate as the CoA ester because it lacks an alternate sink. The Arabidopsis peroxisomal acyl CoA synthetases, LACS6 and 7, exhibit preference for very long-chain substrates and their active sites are located on the matrix side of the membrane

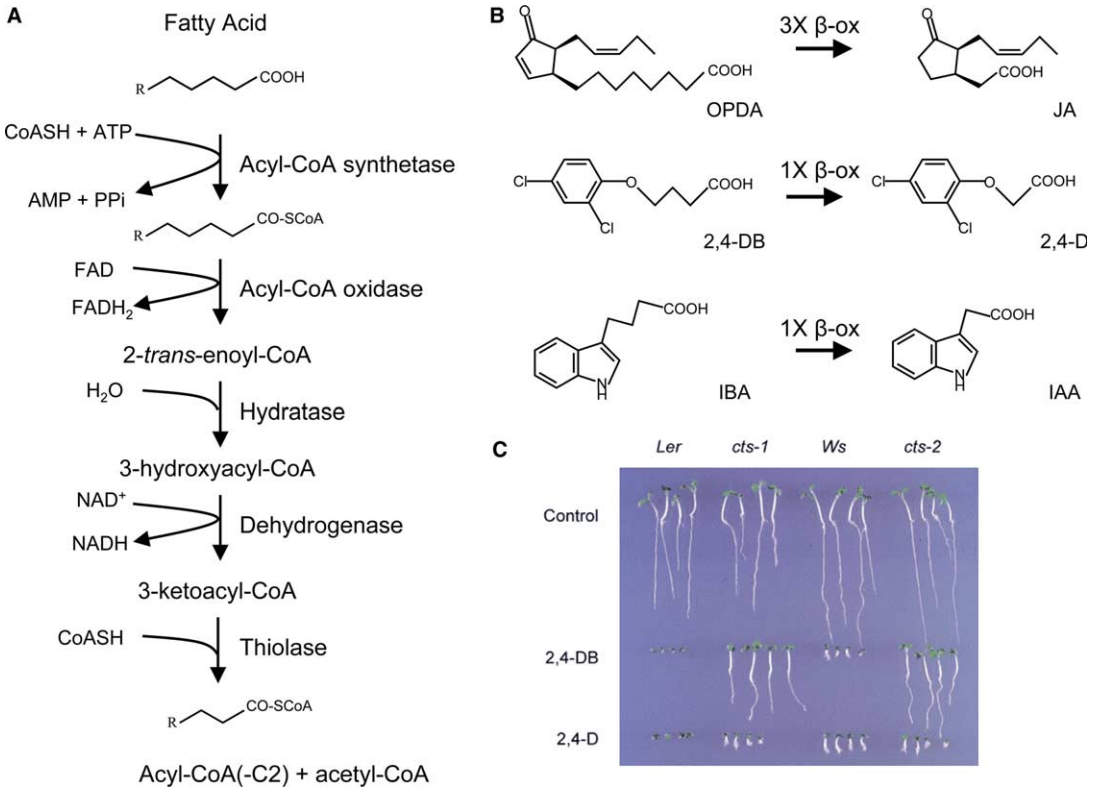

Fig. 3. β-Oxidation of fatty acids and signalling compounds in plants. (A) "Core" β-oxidation of straight chain fatty acids. Substrates are activated by esterification to CoA prior to β-oxidation. Acyl CoA is then converted to a 2-*trans*-enoyl CoA by acyl CoA oxidase and the multifunctional protein catalyses the hydration of 2-*trans*-enoyl-CoA to 3-hydroxyacyl-CoA and subsequent oxidation to 3-ketoacyl-CoA. In the final reaction of β-oxidation, 3-ketoacyl-CoA thiolase catalyses the thiolytic cleavage of 3-ketoacyl CoA to yield acyl CoA and acetyl CoA. This cycle is repeated with the removal of two-carbon units until the fatty acid is completely oxidised. (B) The jasmonate precursor, OPDA, which is derived from the 18-carbon straight chain fatty acid, linolenic acid (18:3), is converted to JA by three rounds of β-oxidation. Similarly, a single cycle of β-oxidation is required for conversion of the auxin precursors 2,4-DB and IBA to 2,4-D and IAA, respectively. Following β-oxidation steps, action of a thioesterase is also required to release the free acids (2,4-D, IAA and JA) from their CoA esters. (C) Whilst wild type plants of both Landsberg *erecta* (L*er*) and Wassilewskija (Ws) ecotypes are sensitive to both 2,4-D and 2,4-DB, *cts* mutants are resistant to 2,4-DB which cannot be oxidised to 2,4-D in these plants. The photograph in panel C is reproduced with permission from reference [111].

[121,122]: therefore CTS may import free FA which are activated in the peroxisome (Fig. 2).

The finding that all fatty acid chain lengths are mobilised in the WT and retained to some extent in *cts* mutants argues for a wide substrate specificity or alternatively for a regulatory role for CTS [111]. Moreover, the resistance of *cts*/*ped3*/*pxa1* mutants to 2,4-DB and IBA suggests that CTS can mediate the uptake of these compounds (or their CoA esters). The concept of CTS as a broad specificity transporter or transport regulator and the requirement for β-oxidation in biosynthesis of the lipid-derived plant hormone, jasmonic acid (JA) led to the discovery that CTS is involved in JA accumulation in Arabidopsis leaves [123]. Both basal and wound-induced JA were markedly reduced in *cts* plants and JA-induced gene expression was compromised. In JA biosynthesis, a cyclopentenone intermediate, 9*S*,13*S*-12-oxophytodienoic acid (OPDA), itself a potent signalling molecule, is synthesised in the chloroplast and transported to the peroxisome where it is reduced and undergoes three cycles of β-oxidation to yield JA (Fig. 3). A model was proposed in which OPDA (or another JA intermediate) is transported into the peroxisome by two parallel pathways: a CTS-dependent route and a passive route involving anion trapping in the alkaline peroxisomal matrix [123]. A potential role for CTS in hormone metabolism points to functions beyond germination and establishment and accordingly, *CTS* is expressed throughout the plant.

A model in which CTS acts as a transporter for a wide range of substrates is reminiscent of multidrug resistance ABC transporters which transport structurally disparate compounds which share only broadly generic chemical properties (e.g., cationic basic compounds; organic anions, etc.). It may be that further potential CTS substrates can be predicted, based on their physicochemical properties. Whilst such a model represents a simple scenario consistent with current experimental data, it is also possible that CTS functions not as an ATP-dependent primary pump but as a transport regulator or possibly in both modes. There are precedents for this amongst ABC transporters of other subclasses, for example: mammalian multidrug resistance P-glycoproteins are both ATP-dependent drug pumps and regulators of volume-activated chloride channels [124]. Similarly, Arabidopsis multidrug resistance associated proteins 4 and 5 not only exhibit pump activity but also regulate stomatal aperture through as-yet unknown mechanisms which probably involve modulation of ion transport activity ([125–127] and see reviews elsewhere in this volume). It will be interesting to

determine whether these concepts also apply to CTS or other ABCD proteins.

4.5. Prokaryotic homologues?

Both *Sinorhizobium meliloti*, a legume symbiont, and *Brucella abortus*, a mammalian pathogen, require the BacA protein to establish intracellular infections in their hosts [128]. BacA does not contain an NBF but shares sequence similarity with the TMD region of the peroxisomal ABC transporters and was found to play a role in the VLCFA content of lipid A in both organisms, suggesting that it is involved in transport of fatty acids, although it is also possible that BacA interacts with homologues of MsbA, a lipid flippase which is responsible for movement of lipopolysaccharide to the periplasmic face of the inner membrane. Whether this has functional implications for eukaryotic ABC transporters is not clear, since the NBF, critical for activity in peroxisomal ABC transporters, is not conserved in BacA. The evolutionary origin of BacA is also an interesting but unresolved question.

5. Conclusions and perspectives

Whilst peroxisomal ABC transporters are present in all eukaryotic genomes, there are important differences in the organisation and functions of the ABCD subfamily in different taxa. It has been noted that orthologous pairs of ABC genes are found much less frequently than in other conserved gene families, and this appears to be the case for peroxisomal ABC proteins [41]. We have attempted to assess the evolutionary relationships of the ABCD subfamily but found that trees of differing topology were supported by similar statistical probabilities, preventing meaningful inferences. However, it is likely that an ancestral half-size protein gave rise to the current spectrum of transporters. An ancestral function is harder to propose: although it is tempting to speculate that peroxisomal import of fatty acids or fatty acyl CoAs could be the function of a prototypical ABCD transporter, phylogenetic analysis of organellar distribution has led to the suggestion that the yeast proteins, Pxa1p and Pxa2p, are originally of mitochondrial origin [129]. The significance of this is unclear: although this might reflect transfer of functions from mitochondria to peroxisomes, this hypothesis is not proven and fatty acids are imported into mammalian mitochondria by a different mechanism involving the concerted action of carnitine palmitoyl transferase I, the carnitine/acylcarnitine translocase and carnitine palmitoyl transferase II [57].

Regardless of the phylogenetic details, the varying functions of peroxisomes suggest that the requirements for transport of substrates across the peroxisomal membrane differ between organisms and that the functions of ABCD proteins are likely to have evolved in concert with the roles of peroxisomes in different organisms (Table 1). Whether β-oxidation is confined to peroxisomes, as in yeast and plants, or is also shared with the mitochondrion, as in animals, may be an important factor. In some organisms, the differential expansion of the ABCD family reflects not only functional diversification but also both tissue specificity, as is the case for the four mammalian peroxisomal ABC transporters. In contrast, although plants are noted for their peroxisomal diversity [130] and contain more ABC proteins in total than other organisms of similar complexity [37], Arabidopsis has only a single peroxisomal ABC protein which is expressed throughout the plant, presumably in different peroxisome subtypes [112,123]. Analysis of knockout mutants has indicated roles in fatty acid catabolism, hormone synthesis and development – this raises the question of how several apparently different roles for this protein are controlled and co-ordinated. One potential answer is that this could be achieved by peroxisomal specialisation, i.e., the predominant function of CTS could be dictated by the complement and substrate-specificity of downstream enzymes in each peroxisome sub-type or possibly by interaction with different membrane proteins.

Although many biochemical details are yet to be determined, models for peroxisomal import of FA appear to differ between organisms (Fig. 2) and the question remains of whether peroxisomal ABC transporters all act as ATP-dependent pumps of lipids and lipid metabolites, or whether they mediate or regulate transport through other mechanisms. The evidence for direct transport is strong in the case of Pxa1p/Pxa2p and PMP70 but less so for ALDP and other ABCD proteins. Unequivocal demonstration of transport will require the reconstitution of peroxisomal ABC proteins into lipid vesicles, a challenging task given the difficulties of purifying membrane proteins, the limited water solubility of putative substrates and the heterodimeric nature of at least some transporters. A further complicating factor is the potential requirement for accessory proteins which may deliver lipophilic substrates to the peroxisome or interact with the transporter on the lumenal side of the membrane. Nevertheless such studies are key to our understanding of the ABCD subfamily and, together with genetic approaches, may also lead to the identification of novel substrates and functions which can only add to the interest of this fascinating group of proteins.

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