Supplementary Material for: The Biochemistry of Headgroup Exchange During Triacylglycerol Synthesis in Canola

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Table S1. Pair-wise percent identity and similarity between BnROD1 proteins and Arabidopsis ROD1 (AtROD1).

	BnROD1.A3	BnROD1.A5	BnROD1.C3	BnROD1.C5	At ROD1
BnROD1.A3		98.2	85.5	86.9	79.9
BnROD1.A5	98.9		86.9	87.2	78.5
BnROD1.C3	91.7	92.7		97.5	80.5
BnROD1.C5	92.1	93.1	99.3		81.1
At ROD1	88.1	88.1	88.4	88.1	
	% Similar				% Identical

Table S2. Pair-wise percent identity and similarity between additional proteins and Arabidopsis ROD1 (AtROD1), with notes.

	XP013676101	XP013682366	XP013714642	XP013717241	At ROD1
XP013676101		97.6	55.7	55.4	72.9
XP013682366	99.3		56.1	55.7	74.7
XP013714642	70.9	70.6		99.6	56.1
XP013717241	70.9	70.6	100		55.8
At ROD1	84.8	84.9	70.1	70.1	
	% Similar				% Identical

Notes:

1. None of the genes encoding these four *B. napus* proteins is expressed in seeds during oil synthesis.

2. The sequences XP013676101 and XP013682366 are similar to AtROD1 but both contain a H247Q substitution. This histidine is part of the active site of this class of cholinephospotransferases in both plants (PDCT) and animals (sphingomyelin synthase) (Lu, et al., 2009).

3. Relative to the other sequences, XP013714642 and XP013717241 are shorter, with truncation of both the N-terminal (34aa) and C-terminal (11aa).

Table S3. Expression of four *BnROD1* genes at six time-points during seed filling in *B. napus*. Data are expressed as target reads per million reads in the database. The means and standard errors are shown for three independently grown and harvested samples.

Gene	15daf	15daf-SE	21daf	21daf-SE	28daf	28daf-SE	32daf	32daf-SE	37daf	37daf-SE	50daf	50daf-SE
BnROD1.A3	12.26	1.05	28.07	6.53	13.23	0.87	8.42	0.38	6.90	3.31	0.00	0.00
BnROD1.C3	6.61	0.54	26.07	1.23	20.49	1.43	12.82	1.20	6.56	2.98	0.00	0.00
BnROD1.A5	2.57	0.19	0.25	0.05	0.27	0.11	0.05	0.05	0.33	0.01	0.00	0.00
BnROD1.C5	0.55	0.28	0.15	0.08	0.05	0.05	0.22	0.14	0.06	0.06	0.00	0.00

Methods: At three different times *B. napus* plants were grown in a growth chamber at 18°C with 16 h of light (400 µE) and a relative air humidity of 60%. From first day of flowering, flowers were tagged for the determination of seed developmental stages. At different developmental stages seed samples were collected for the three biological replicates, snap frozen, and stored at -80°C. Total mRNA from seeds of the three biological replicates were isolated according to standard methods. Gene expression was measured using transcript profiling (RNA-seq) by Illumina HiSeq paired-end sequencing (2x100 bp sequences). The counts per transcript (transcript per million; tpm) were determined as described by Li and Dewey (Li, B. and Dewey, C. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12: 323).

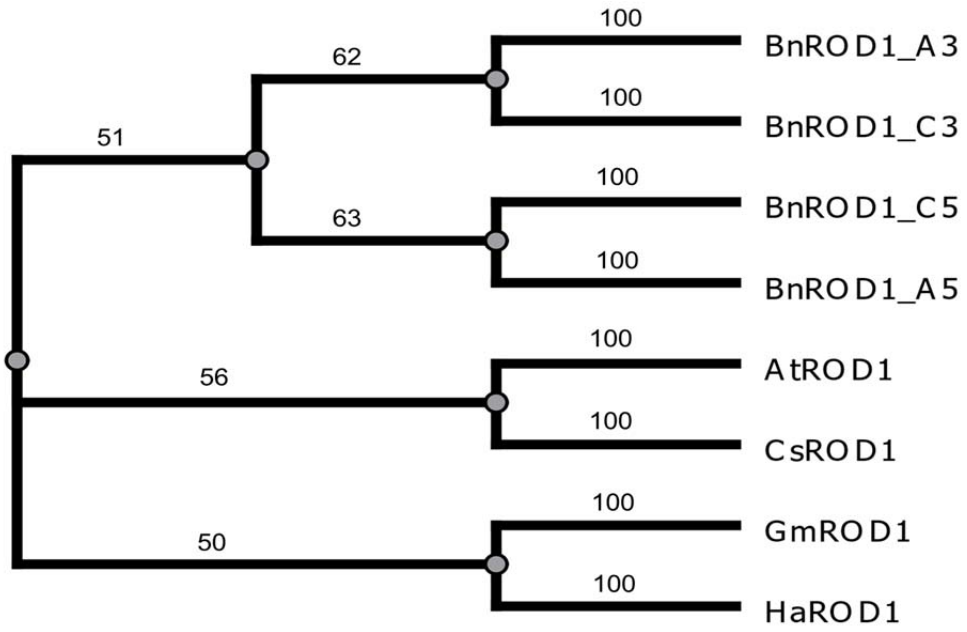

Figure S1. Cladogram showing the implied phylogeny of eight ROD1 proteins.

Methods: The protein sequence for BnROD1.A3, BnROD1.A5 BnROD1.C3 BnROD1.C5, AtROD1, CsROD1, GmROD1, and HaROD1 where aligned using the ClustalW algorithm. The alignment was used to generate a phylogenic tree with PHYLIP Neighbor Joining and a Jones-Taylor-Thornton distance matrix model calculated using Unipro UGENE bioinformatics software (Felsenstein, J. (1996). Inferring phylogenies from protein sequences by parsimony, distance, and likelihood methods. Methods Enzymol. 266:418-27.). Numbers indicate bootstrap values. Bn, *Brassica nap*us; At, *Arabidopsis thaliana*; Cs, *Camelina sativa*; Gm, *Glycine max*; Ha, *Helianthus annuus*.

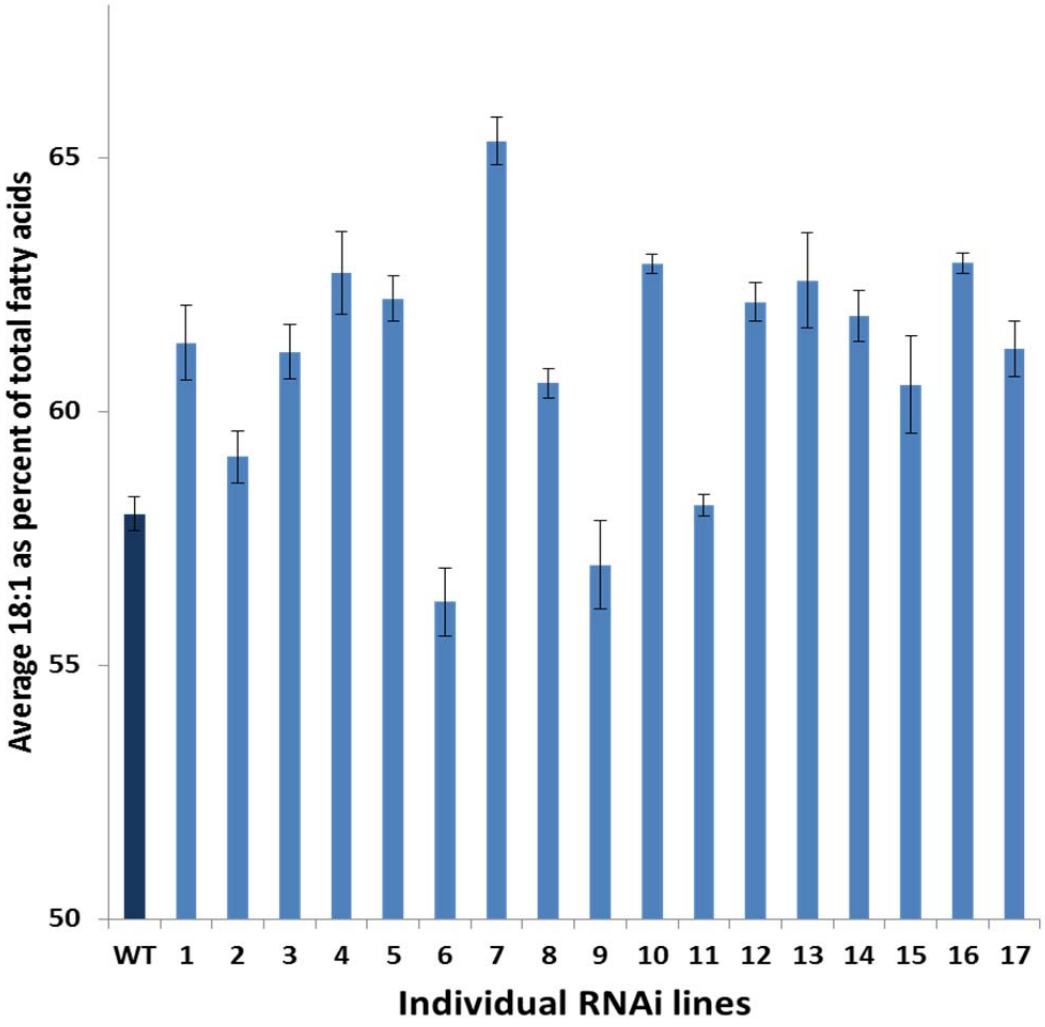

Figure S2. Seed 18:1 content in 17 RNAi lines. Data are for samples from bulk, segregating seed of each T1 line expressing hairpin constructs targeting the *BnROD1.A3* and *.C3* genes (lines 1-9) or the *BnROD1.A3, .C3, .A5* and *.C5* genes (lines 10-17), compared to the wild-type control. Mean +/- SE (n=5).

Figure S3. Protein sequence alignment of PDCT enzymes from *B. napus* (BnROD1.A3 and BnROD1.C3) and Arabidopsis (AtROD1). Residues contributing to the active site are shown in blue. Six putative transmembrane sequences are underlined in green. Sites of the mutations included in Figure 7 are highlighted in red.