Quizzing the Factories of Oilseeds

What Part of 'No' Don't We Understand?

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Training and Outreach:

13 Postdocs 4 Graduate Students 4 Technicians

8 Collaborators 5 REU Students, 6 Honors and Undergraduate

Convergent research identifies limitations and provides solutions

Oils are Attractive Targets for Metabolic Engineering

- Substantial amount of plant oil already used for non-food applications.
- Chemical industry is familiar with fatty acid chemistry and applications.
- Some 1,000 different fatty acid structures occur in nature. Green Chemistry.
- Need modify only one or a few genes?
- Expression in crop plants utilizes agricultural infrastructure.

Evolved Biochemistry, Valuable Oils

Hydroxy from Castor

HFA

Conjugated from Momordica

CFA

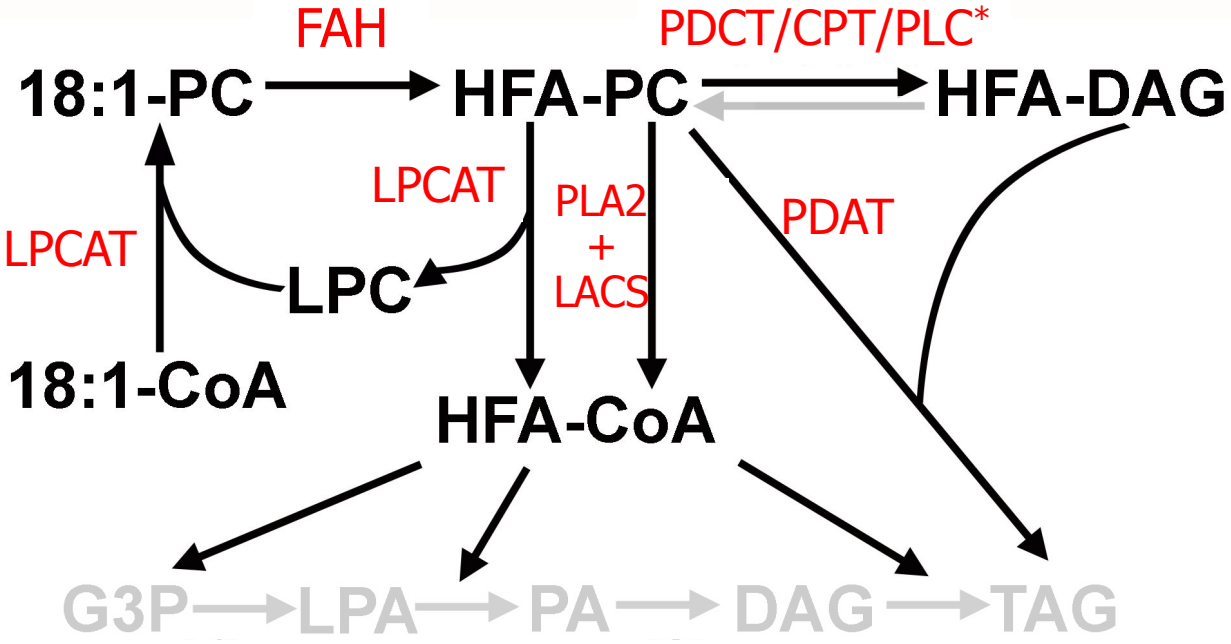
Cyclopropane from Cotton

CpFA

Unusual Fatty Acids in Transgenic Plants

Fatty Acid	Level in source	Level in transgenic
Hydroxy	**90%**	**17%**
Petroselinic	85%	<10%
16:1 Δ6	80%	<10%
Conjugated	**65%**	**13%**
Cyclopropane	**40%**	**< 5%**
Acetylenic	70%	25%
Epoxy	60%	15%
Lauric (+10:0)	65%(+25%)	50-60%

Alternative Paths to HFA-TAG



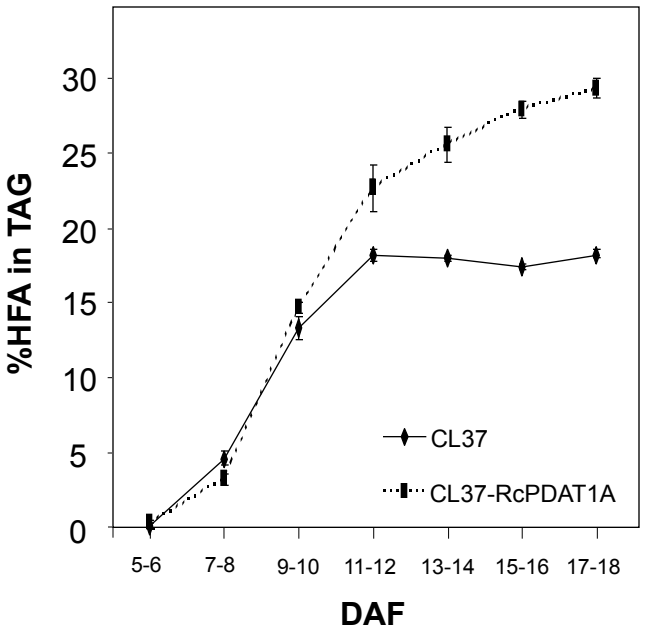
*or PLD+PAP

Promising Results For Two Genes

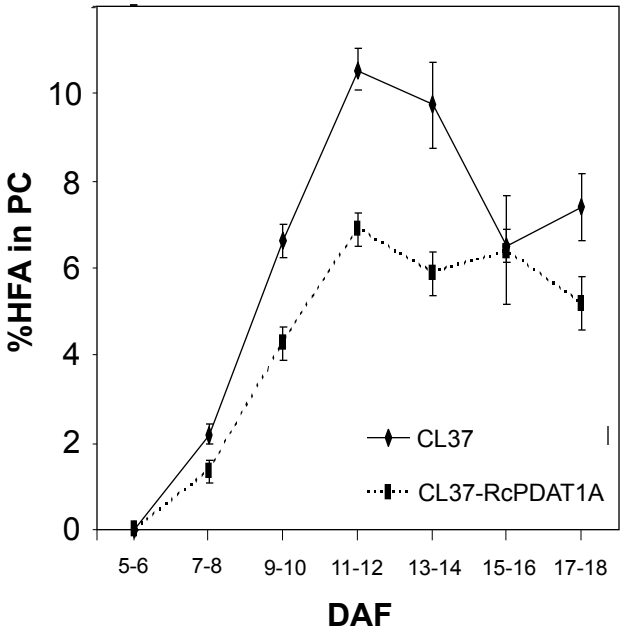
Plant Name	16:0	18:0 + 18:1	18:2	18:3	20:1	18:1-OH	18:2-OH	Total OH
RcDGAT2 #101	17.7	26.6	25.7	7.4	2.0	19.1	1.5	**20.6**
RcDGAT2 #102	15.5	29.7	23.0	6.7	1.6	21.0	2.5	**23.5**
RcDGAT2 #103	14.1	34.1	20.2	5.8	1.6	21.1	3.1	**24.2**
RcDGAT2 #104	14.2	34.8	20.1	5.8	1.7	20.6	2.8	**23.4**
RcDGAT1 #101	16.0	32.9	23.1	6.8	1.5	17.5	2.1	**19.6**
RcDGAT1 #102	14.3	38.2	21.9	5.7	1.4	15.9	2.6	**18.5**
RcDGAT1 #103	16.8	34.6	23.3	6.4	1.5	15.4	2.0	**17.4**
RcDGAT1 #104	16.1	35.9	23.3	5.9	1.4	15.3	2.0	**17.3**
RcDGAT1 #105	14.6	37.0	21.0	6.4	1.5	16.9	2.5	**19.5**
RcDGAT1 #106	15.6	37.2	20.6	6.3	1.8	16.4	2.1	**18.5**
RcLPAAT1 #1	14.3	40.7	17.8	6.4	1.5	16.6	2.6	**18.4**
RcLPAAT1 #2	13.4	36.6	18.2	7.1	8.2	13.6	2.9	**18.2**
RcLPAAT1 #3	13.5	40.3	18.3	6.9	1.5	16.4	2.9	**18.1**
RcPDAT1A-C	11.2	35.5	21.8	7.1	0.5	19.4	4.5	**23.9**
RcPDAT1A-D	11.8	35.1	21.6	7.2	0.5	19.3	4.4	**23.8**
RcPDAT1A-E	11.5	35.1	21.2	6.2	0.5	20.9	4.6	**25.5**
RcLACS4 A	17.8	36.5	23.9	6.1	0.5	15.0	0.2	**15.2**
RcPDAT1B	15.5	35.4	24.7	7.6	1.4	13.0	2.5	**15.5**
CL7	12.5	44.0	19.9	5.6	1.4	12.7	3.8	**16.5**
CL37	16.0	37.3	22.4	5.5	0.4	15.2	3.1	18.3

RcPDAT Reduces HFA in PC

75% More HFA

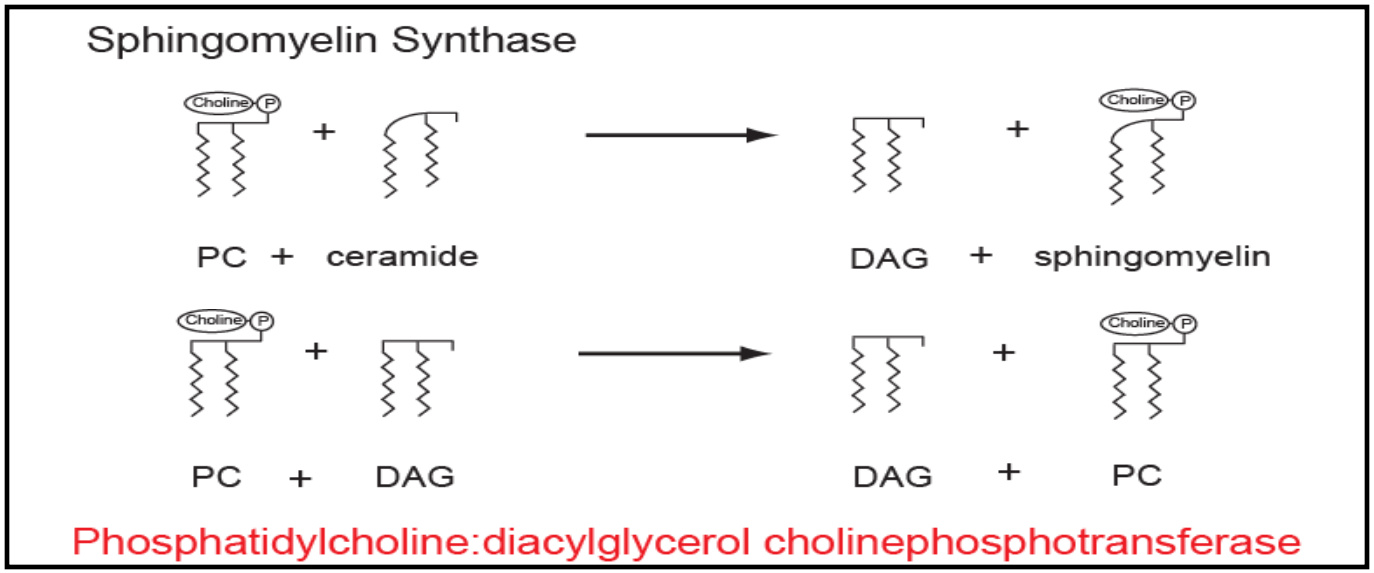


Lower HFA in PC



A New Enzyme of TAG Synthesis

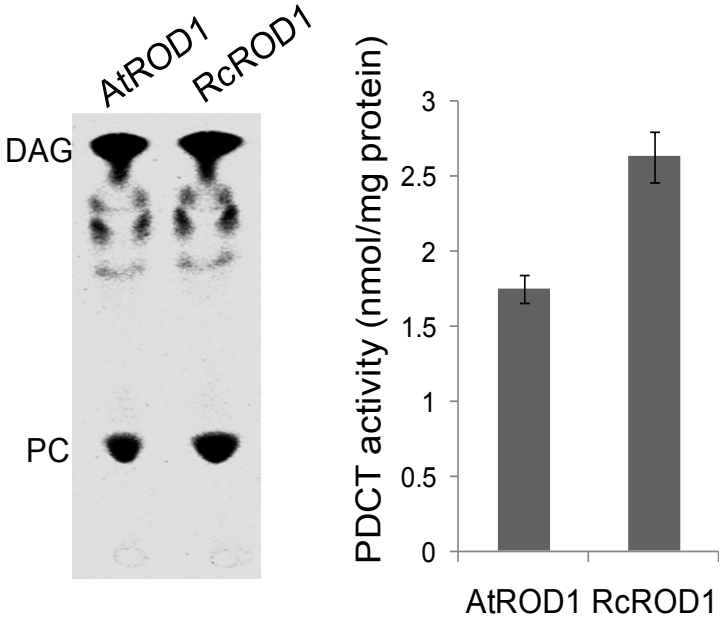
	Mol % of fatty acids in seeds					
	16:0	18:0	18:1	18:2	18:3	20:1
WT	8.4	3.1	15.1	29.2	19.9	18.6
rod1	8.5	3.3	32.8	13.8	15.6	20.6



40% of 18:2+18:3 flux through PDCT

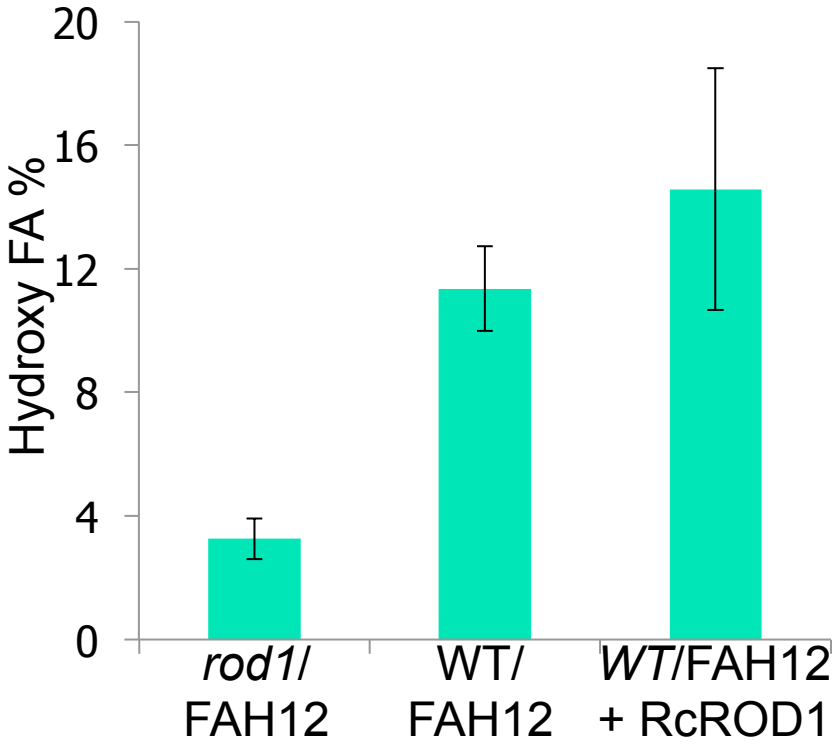
ROD1 and HFA Production

Castor RcROD1 also encodes PDCT activity

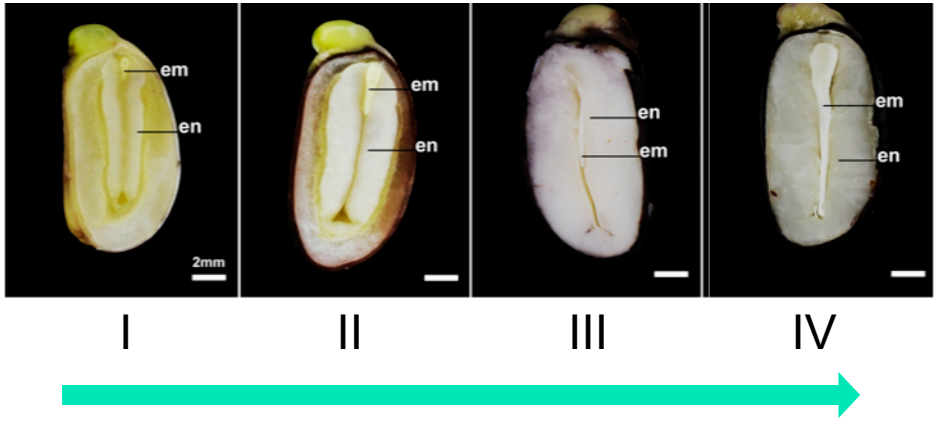


Yeast assays

ROD1 is Required for HFA accumulation in transgenics

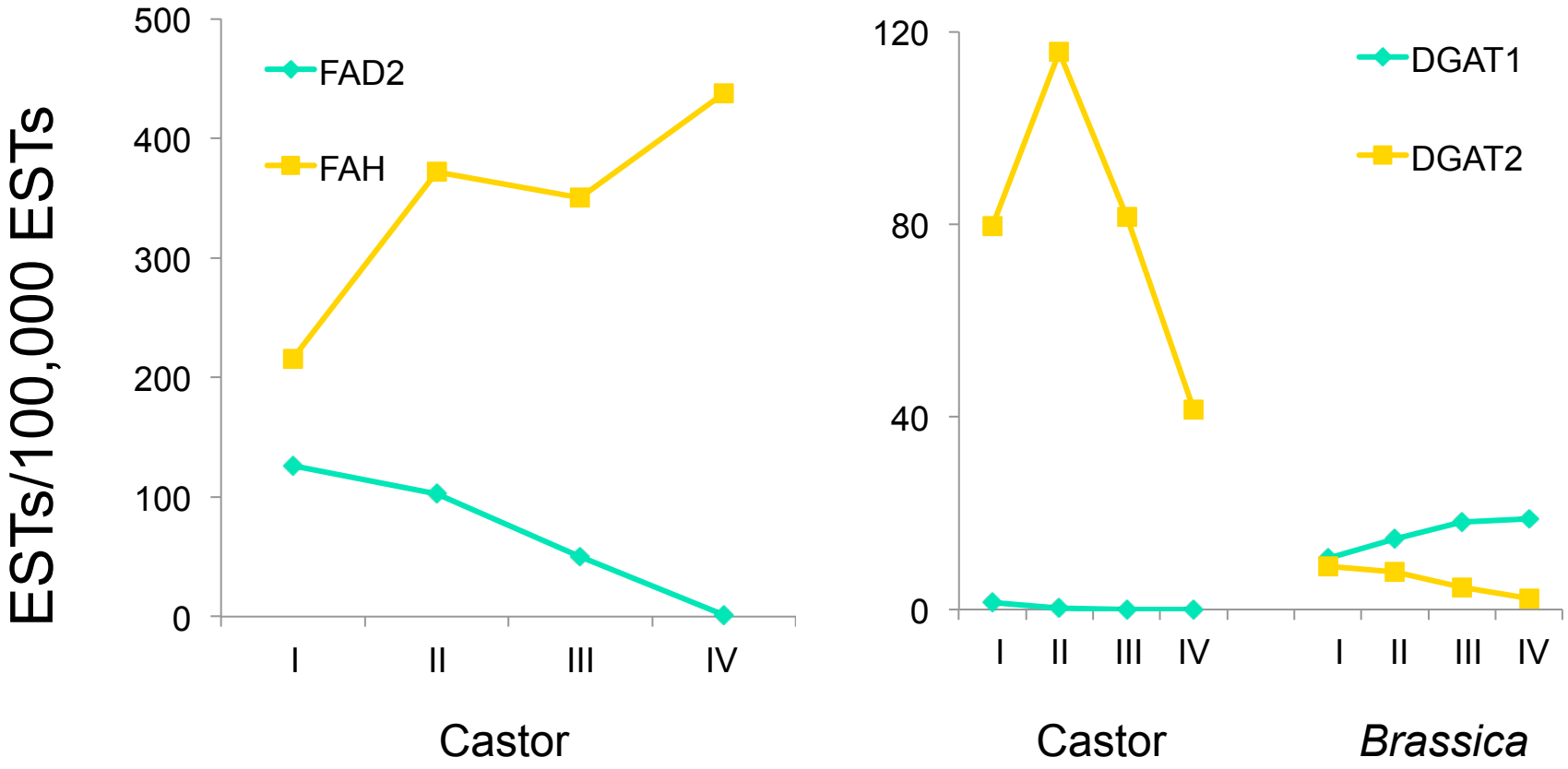


Deep Transcriptional Profiling Provides Key Leads

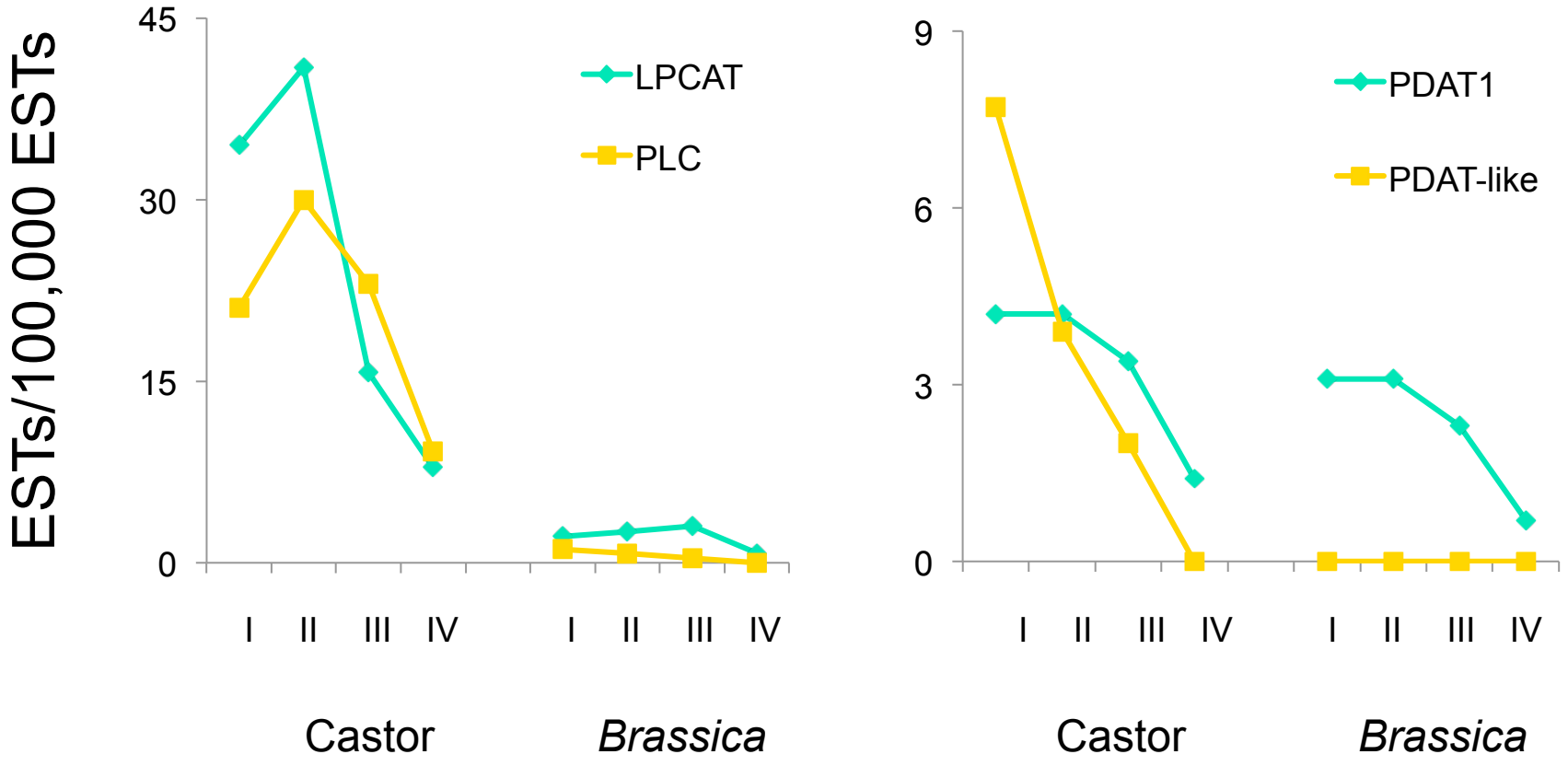


- 454 FLX pyrosequencing
- 1,059,000 ESTs
- Leveraged with other oilseeds

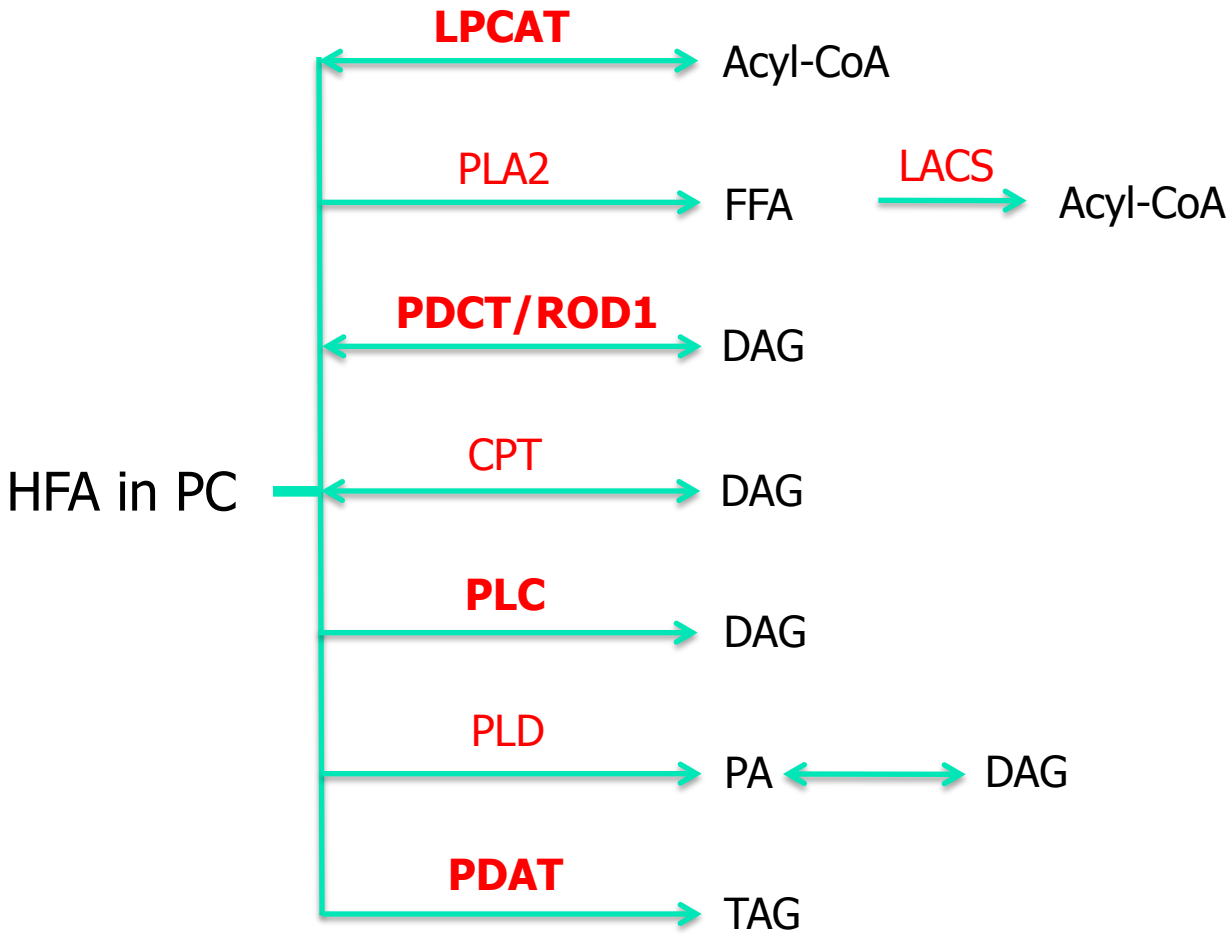
Hydroxylase is Massively Expressed



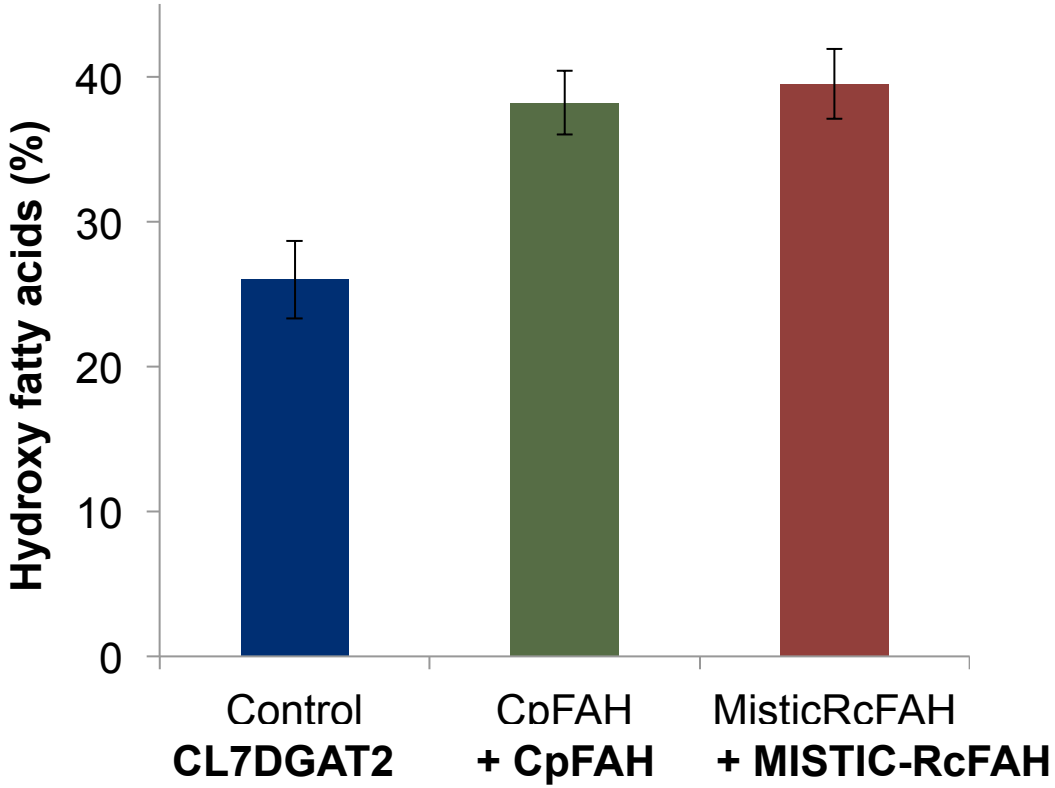
Removing HFA from PC



Seven Routes to Remove HFA from PC

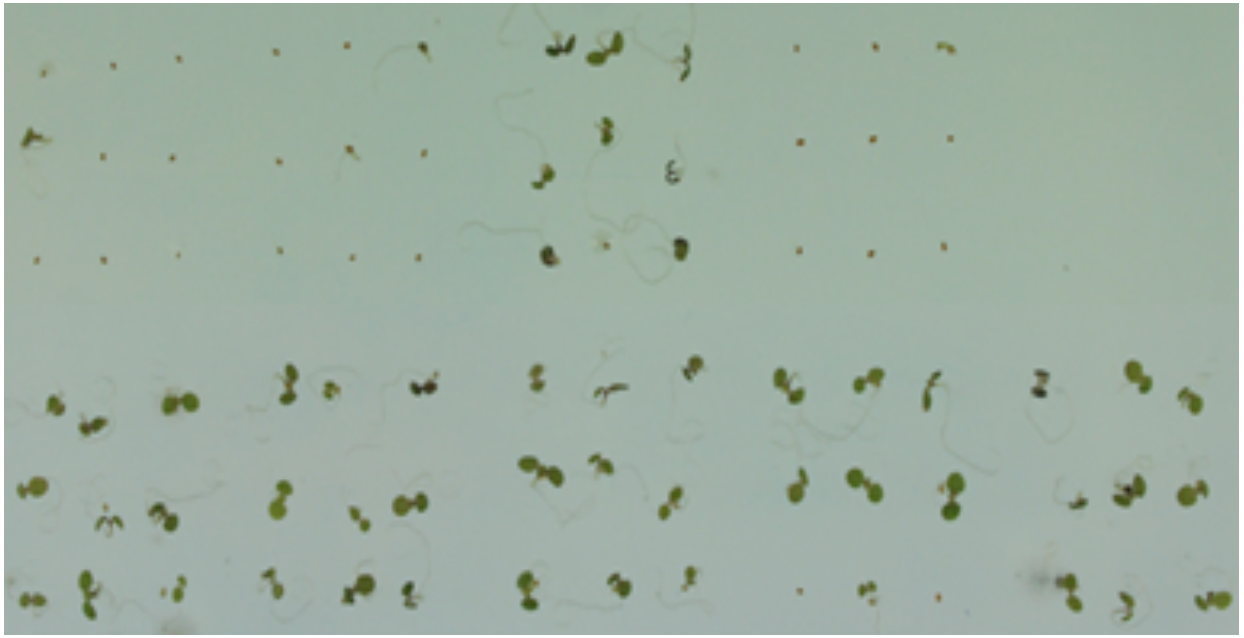


FAH Expression *Is* Limiting



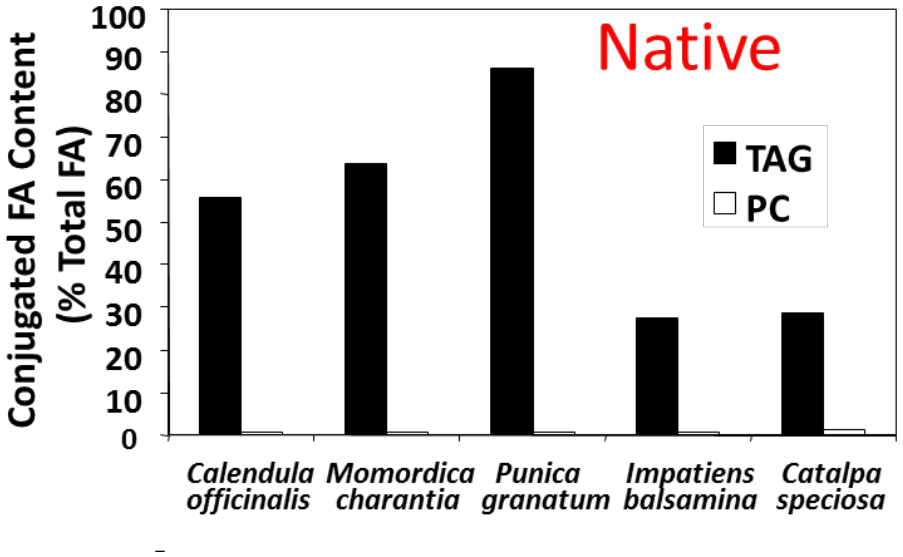
Adding *Claviceps* (Cp) FAH or *Ricinus* (Rc) FAH in CL7DGAT2 increases HFA content from 27% to ~40%

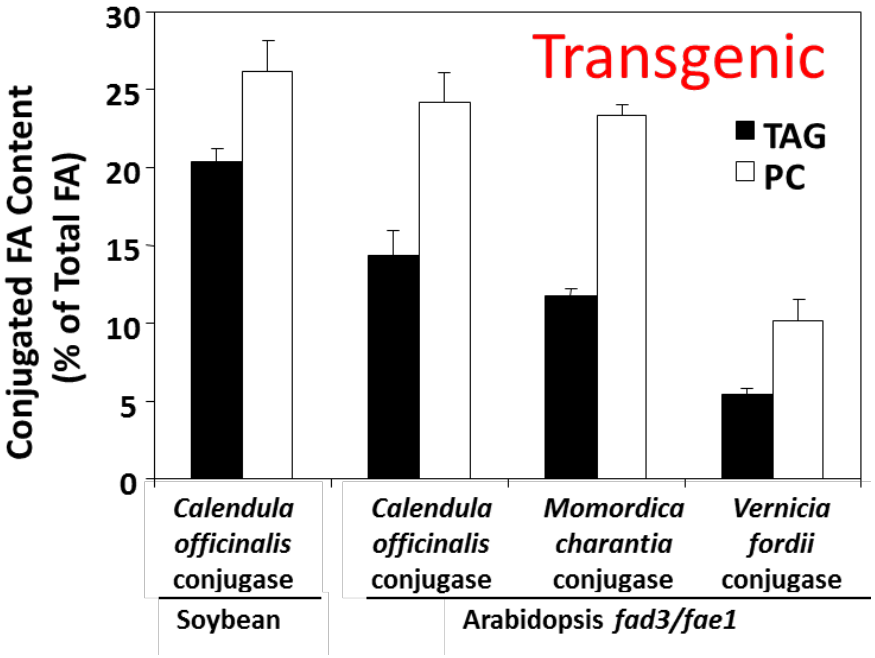
The MISTIC Tag Rescues Germination



- All transformed seeds have approximately 40% HFA
- Representative germination of *FAH12*:CL7DGAT2 seeds transformed with an additional FAH as indicated

Conjugated Fatty Acids Also Accumulate in PC





Soybean

Wt +Conjugase

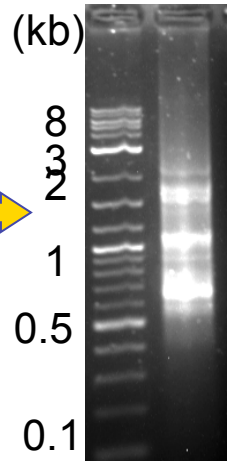
Arabidopsis

Wt +Conjugase

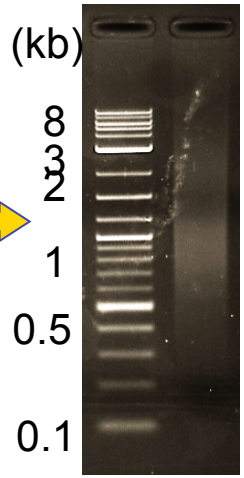
Normalization Strategy

- Unnormalized and Normalized libraries
- 454 deep sequencing

RNA from 18-day-old seeds



Unnormalized cDNA library



Normalized cDNA library

228,000 clean reads

342 lipid genes

Conjugase gene highly expressed (248/87)

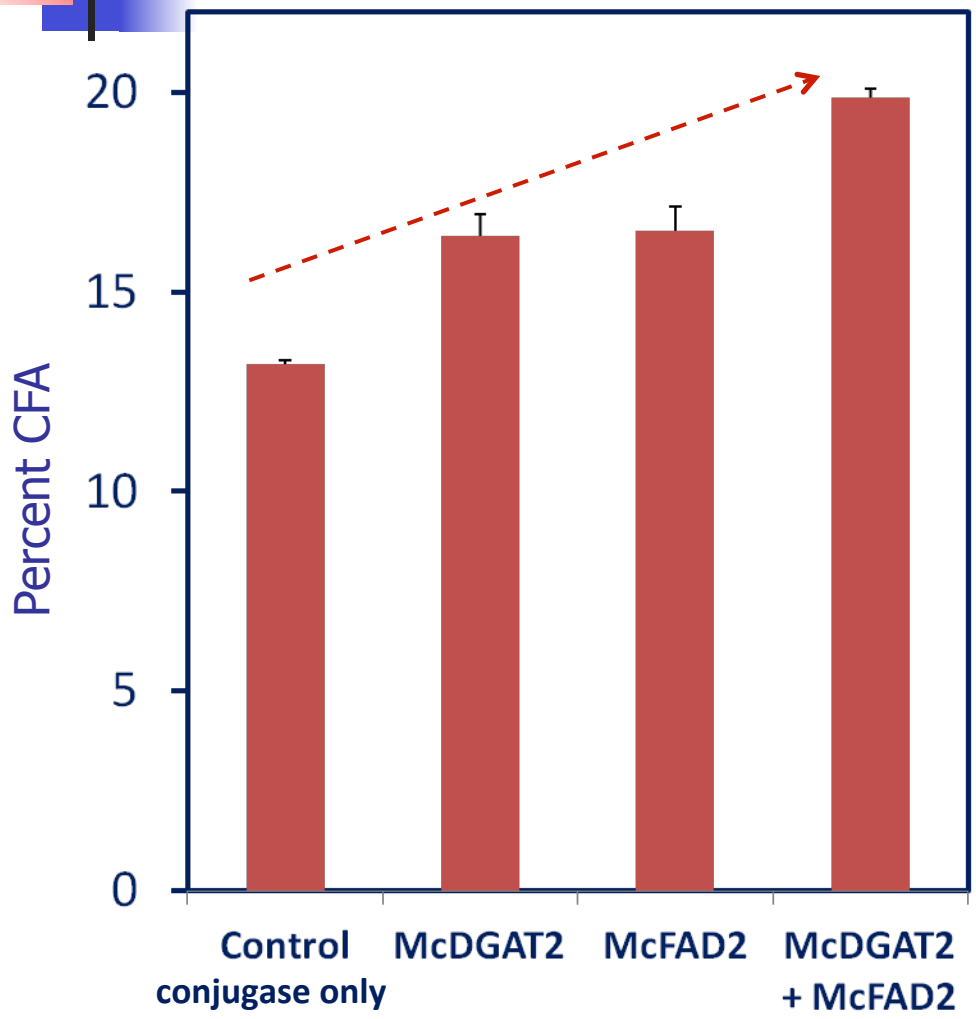
LPCAT top candidate for CFA from PC (29/22)

PLC, PLD and *PDAT* also candidates

ROD1 **not** detected

DGAT1 > *DGAT2*

Initial Successes



conjugase alone

12%

↓

+ DGAT2

or

+ FAD2

16%

↓

+ DGAT2 + FAD2

20%

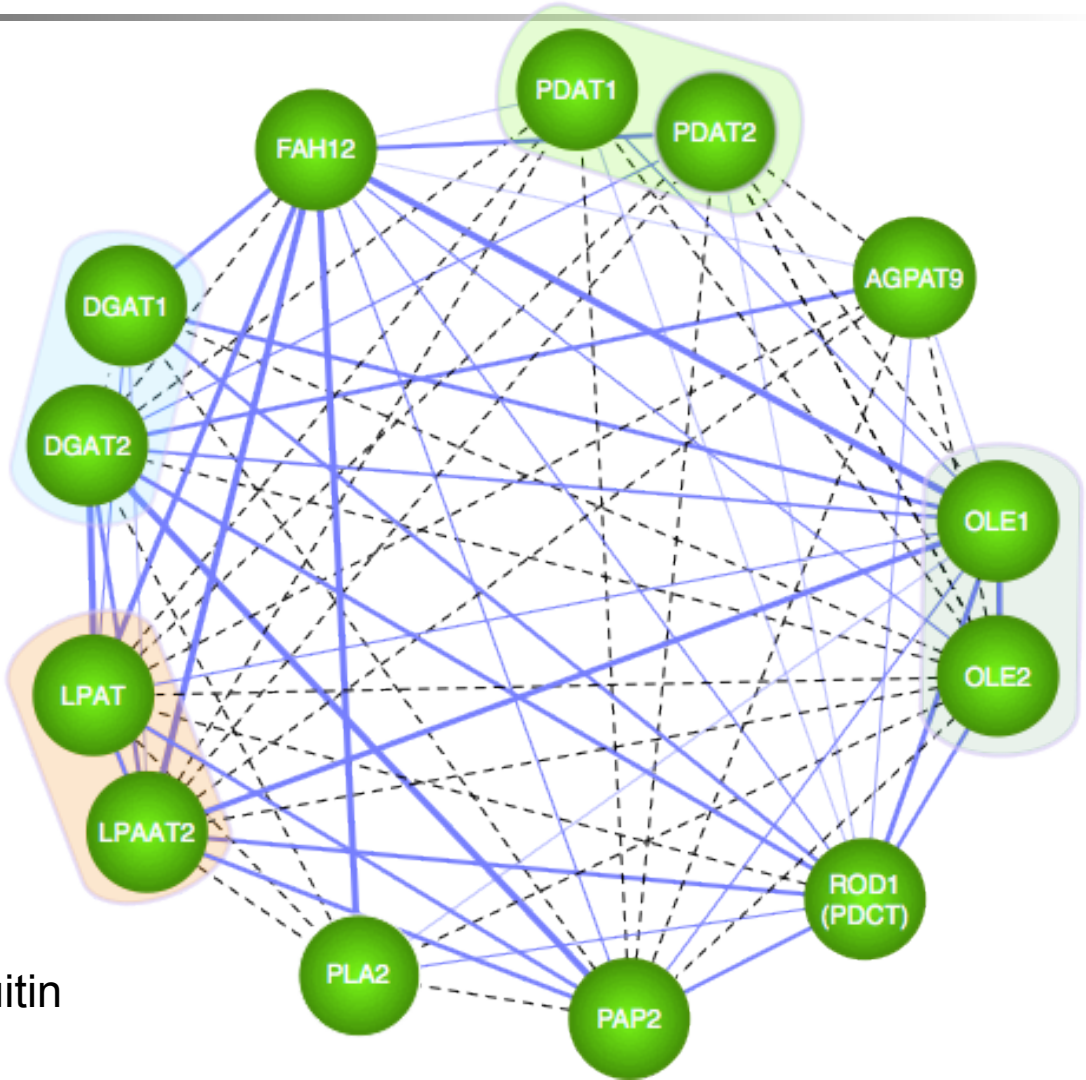
Protein Interactions Connect FAH To Other Pathway Components

Interaction

Strong

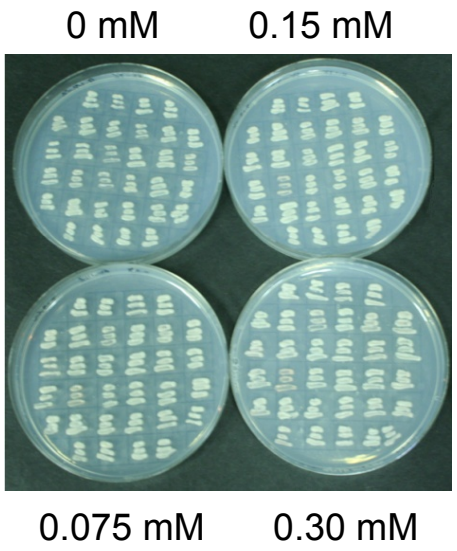
Weak

No interaction

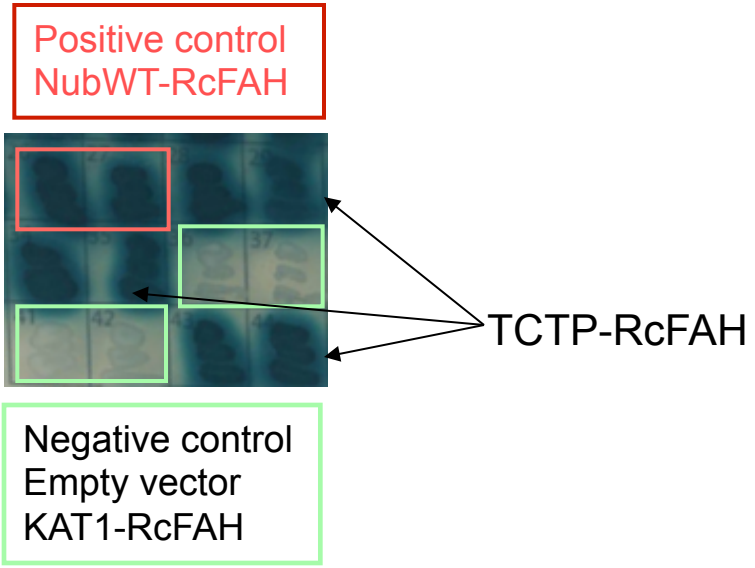


Assay: mating-based split-ubiquitin

TCTP: A Novel FAH-Interactor



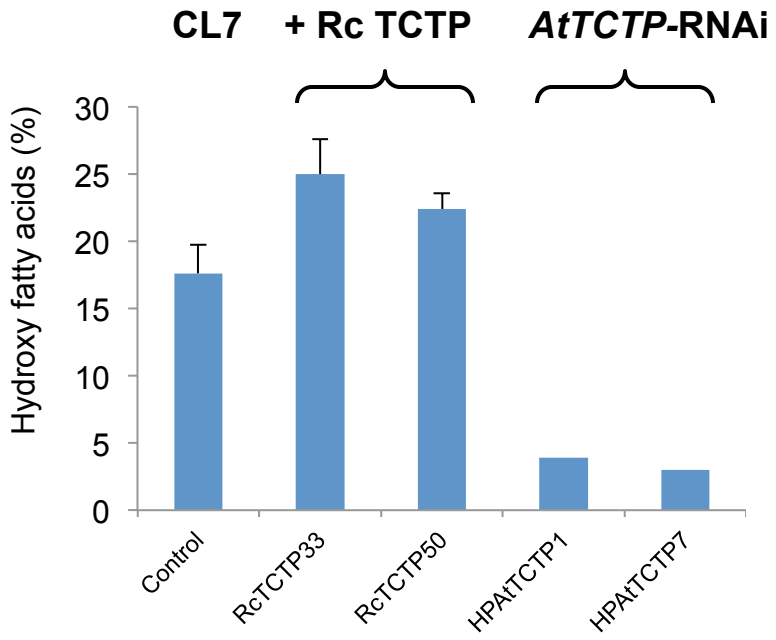
Growth on Methionine demonstrate strong interaction



X-GAL confirms specific TCTP interaction

(TCTP = translationally-controlled tumor protein)

A New Player in HFA Accumulation



- RcTCTP increases HFA
- *AtTCTP*-RNAi strongly reduces HFA

Conclusions

- Modified oils are valuable, but not a ‘one-gene’ enterprise
- We have developed the biochemical knowledge and genetic tools needed to succeed
- MISTIC tag bypasses ER import machinery to maximize protein expression
- Specialized isozymes (for PC metabolism, acyl transferases) are critical components
- Ultrasequencing identifies candidates; biochemistry and transgenics demonstrate their utility
- Cellular context is important (e.g. MISTIC); protein-interaction assays lead to more discoveries
- Integration of our discoveries is now the key to success