The preferred acyl chain donor of the yeast tafazzin

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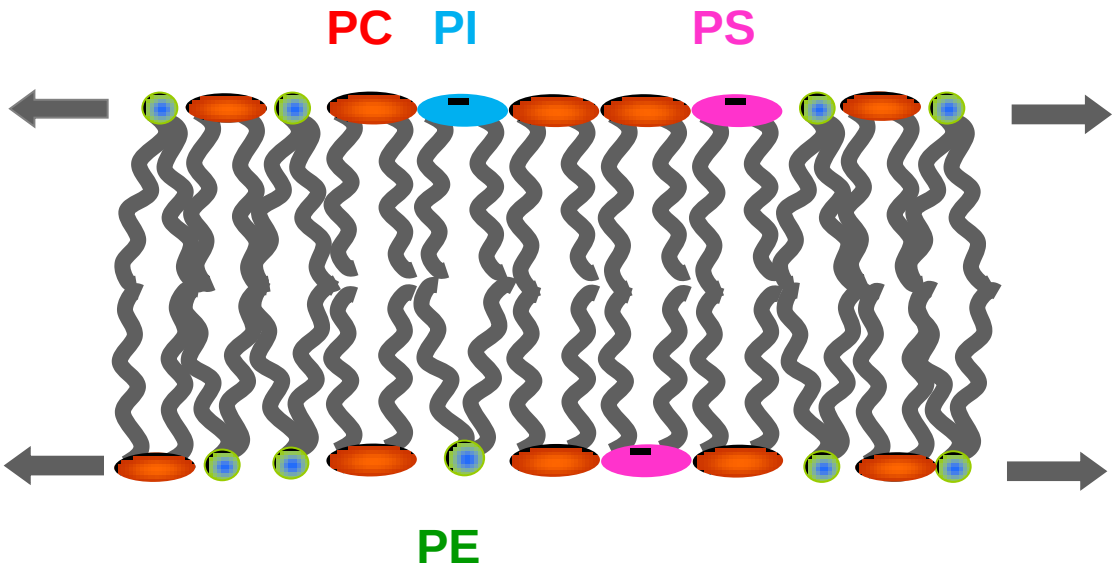
Regulation of membrane lipid homeostasis

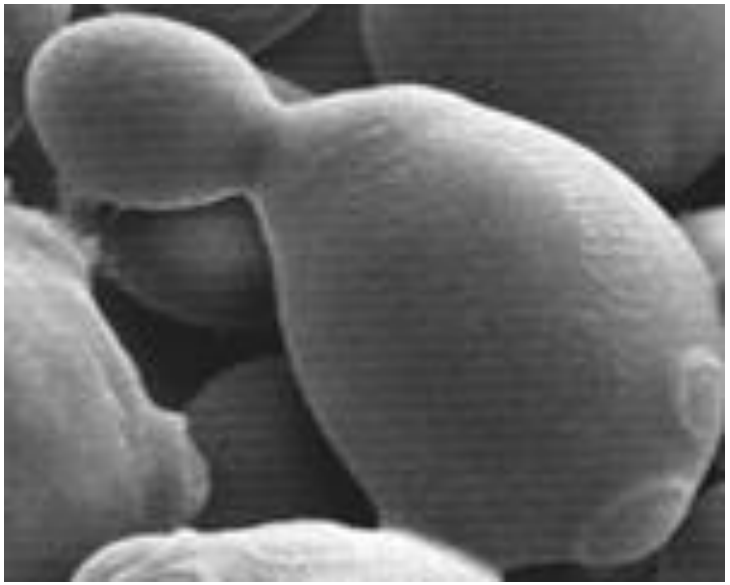
The interplay between phospholipid class and acyl chain composition determines physical properties of the membrane



- Membrane fluidity: **UFA/SFA**
- Membrane thickness: **acyl chain length**
- Membrane surface charge: **% PL^-**
- Membrane intrinsic curvature: **bilayer *vs.* non-bilayer lipids**

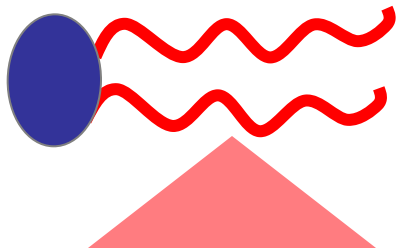
Why do we study regulation of membrane lipid homeostasis in *S. cerevisiae*?

- Phospholipid biosynthetic pathways and membrane lipid composition similar between yeast and higher eukaryotes
- Ease of manipulation
- Limited repertoire of acyl chains
- Tolerance to variation in lipid composition



Phospholipid and **acyl chain** composition of wild type yeast

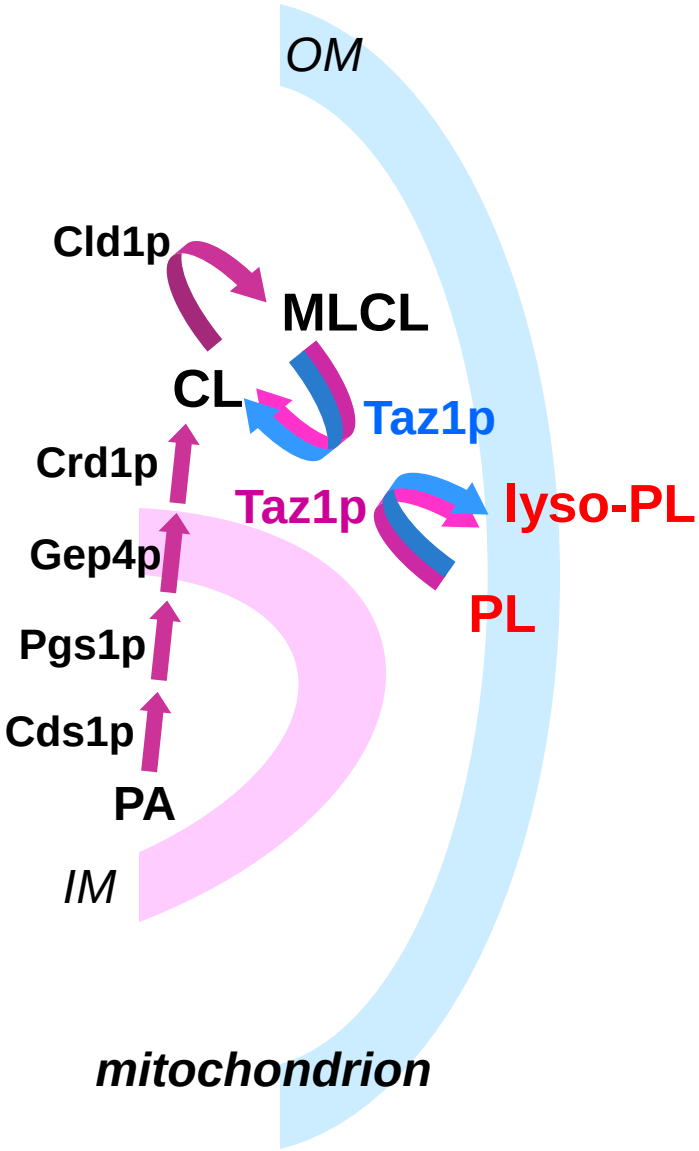
phospholipid class	mol%
phosphatidylcholine (PC)	41
phosphatidylethanolamine (PE)	26
phosphatidylinositol (PI)	18
phosphatidylserine (PS)	9
cardiolipin (CL)	5

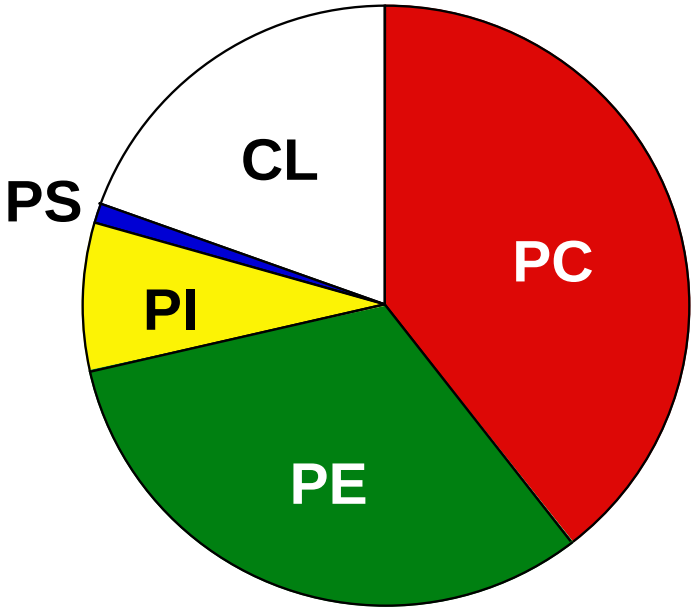


fatty acid	mol%
C14	2
C16:0	15
C16:1	48
C18:0	4
C18:1	30

BY4742 cultured on semi-synthetic lactate medium to mid-log phase

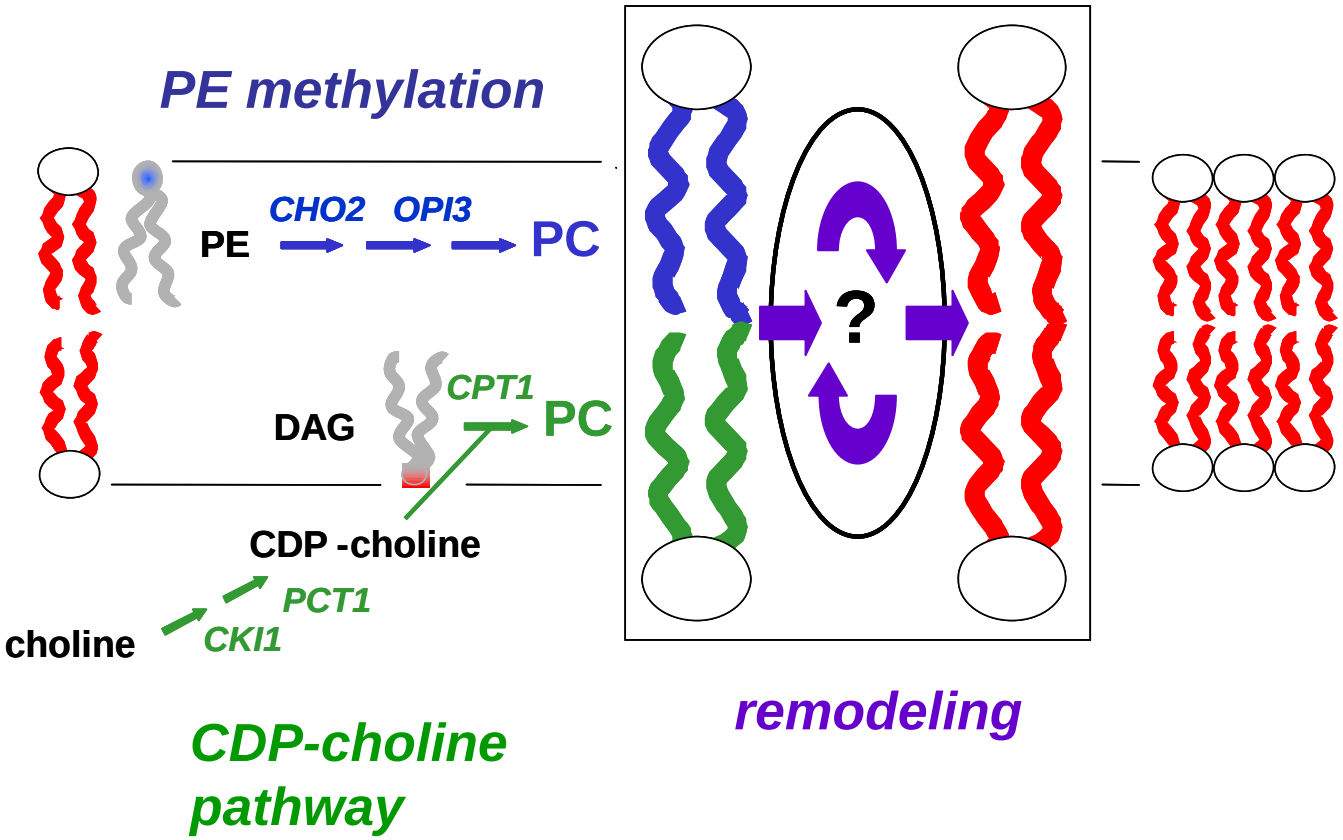
Candidate acyl chain donors of Taz1p





typical PL composition of yeast mitochondria (% of PL-Pi)

Acyl chain exchange/remodeling contributes to the molecular species profile of PC in yeast



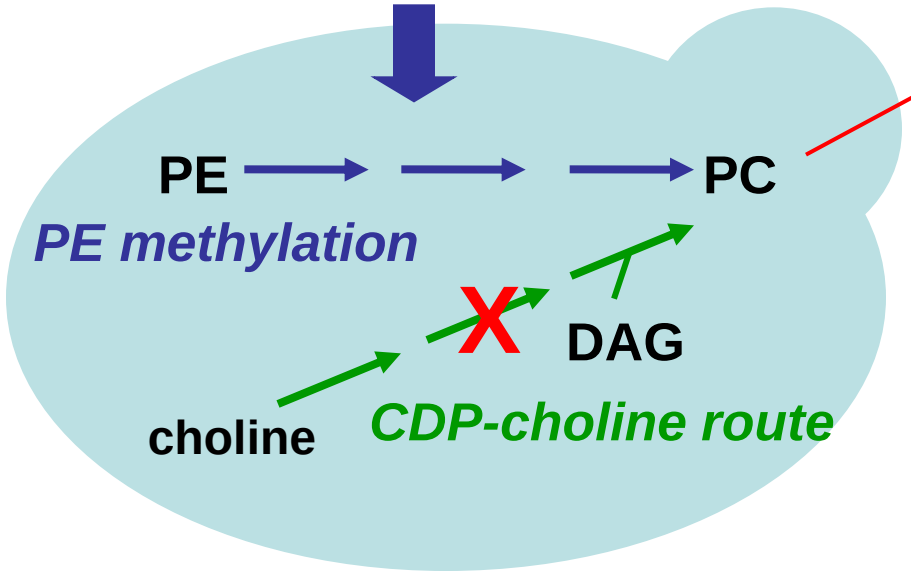
Boumann et al., 2003

PC remodeling is monitored in a *pct1Δ* strain in pulse-chase experiments with stable isotope labeling and detection by ESI-MS/MS

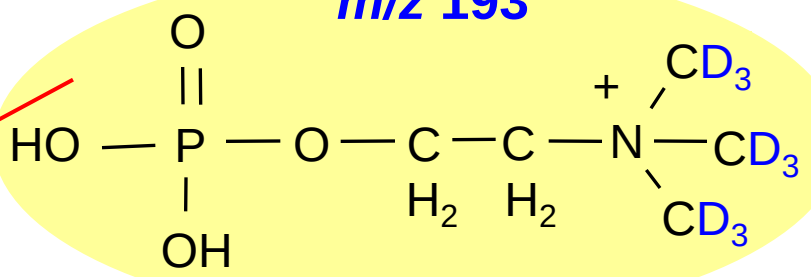
"dynamic lipidomics"

parent ion scan for:

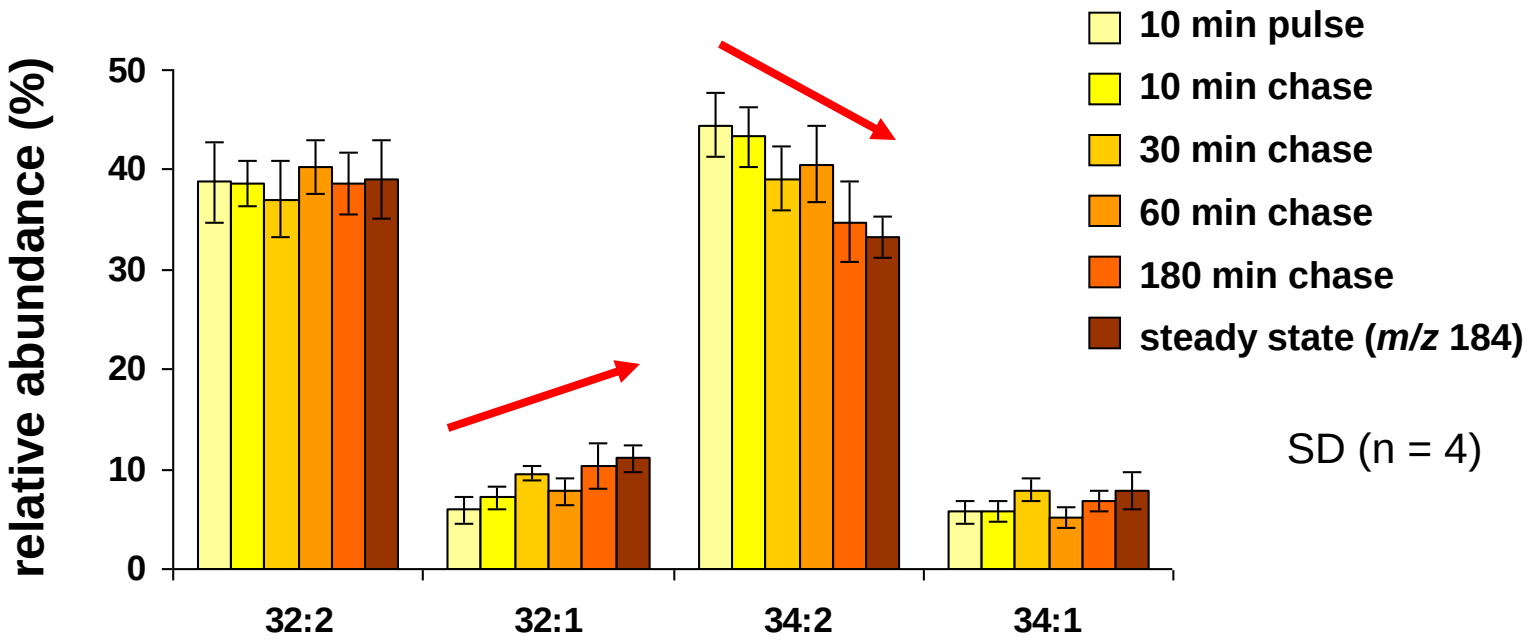
(*methyl*-D_3)-methionine

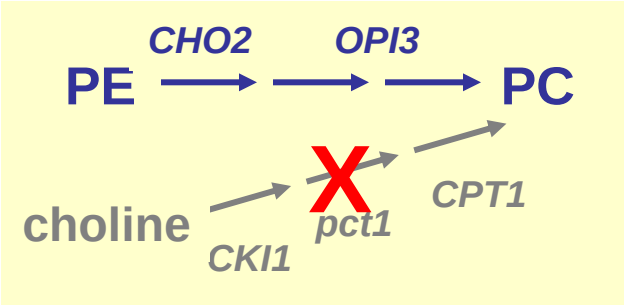


***m/z* 193**



Remodeling by acyl chain exchange contributes to the PC species profile in a *pct1Δ* strain

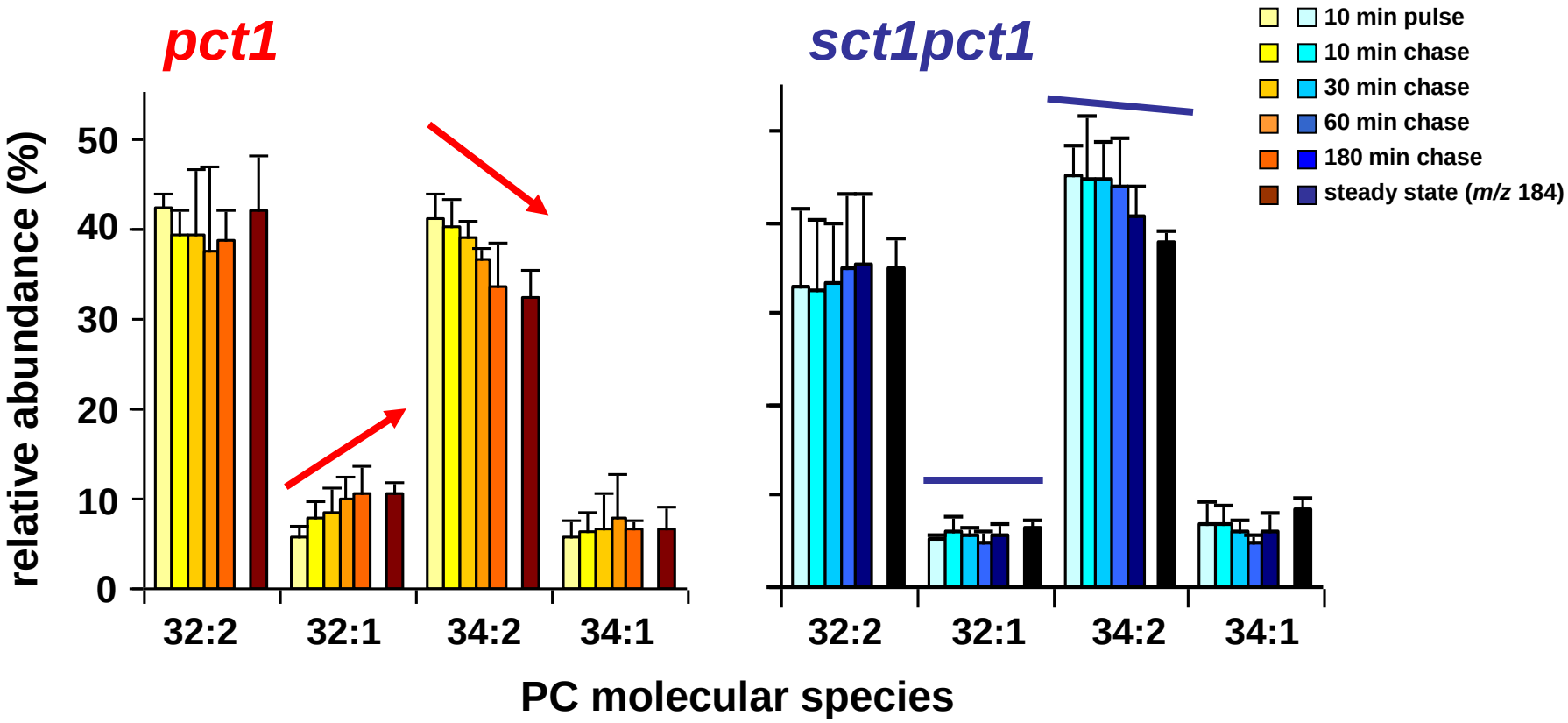




PC molecular species

Cells were pulsed for 10 min with **(*methyl*-D_3)-methionine**, the label was chased with (*methyl*-H_3)-methionine, detection by parent ion scan for ***m/z* 193**

Deletion of the *SCT1/GAT2* gene reduces the extent of remodeling of PC in *pct1Δ* cells



Cells were pulsed for 10 min with **(*methyl*-D_3)-methionine**, the label was chased with (*methyl*-H_3)-methionine

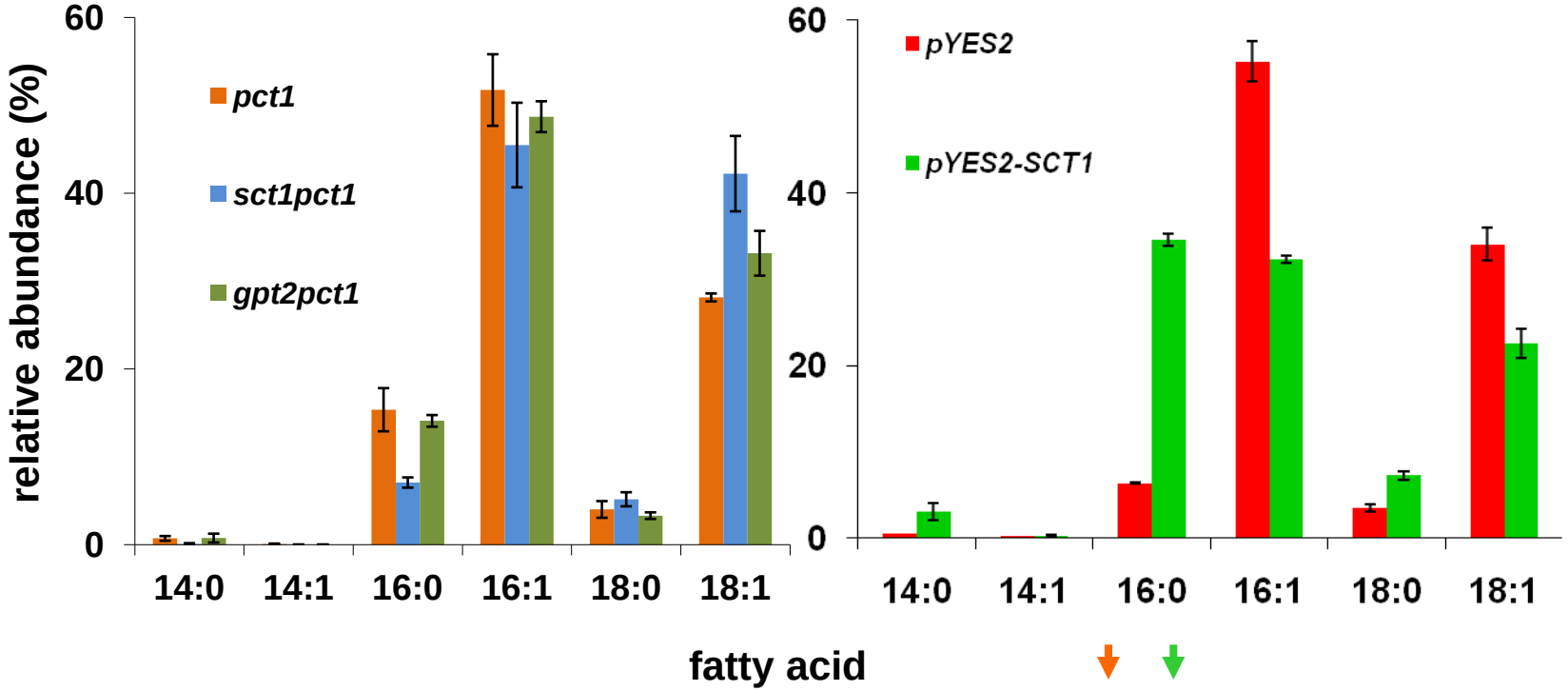
SCT1 (GAT2)

- Encodes a G-3-P/DHAP acyltransferase responsible for attaching an acyl chain at the *sn*-1 position to yield lyso-PA

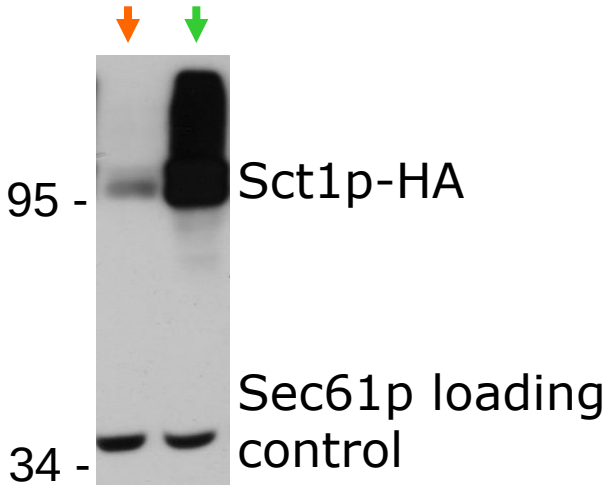
Zheng and Zou, 2001

C16:0 content is decreased by deleting *SCT1*

and 4-fold increased upon overexpression of Sct1p

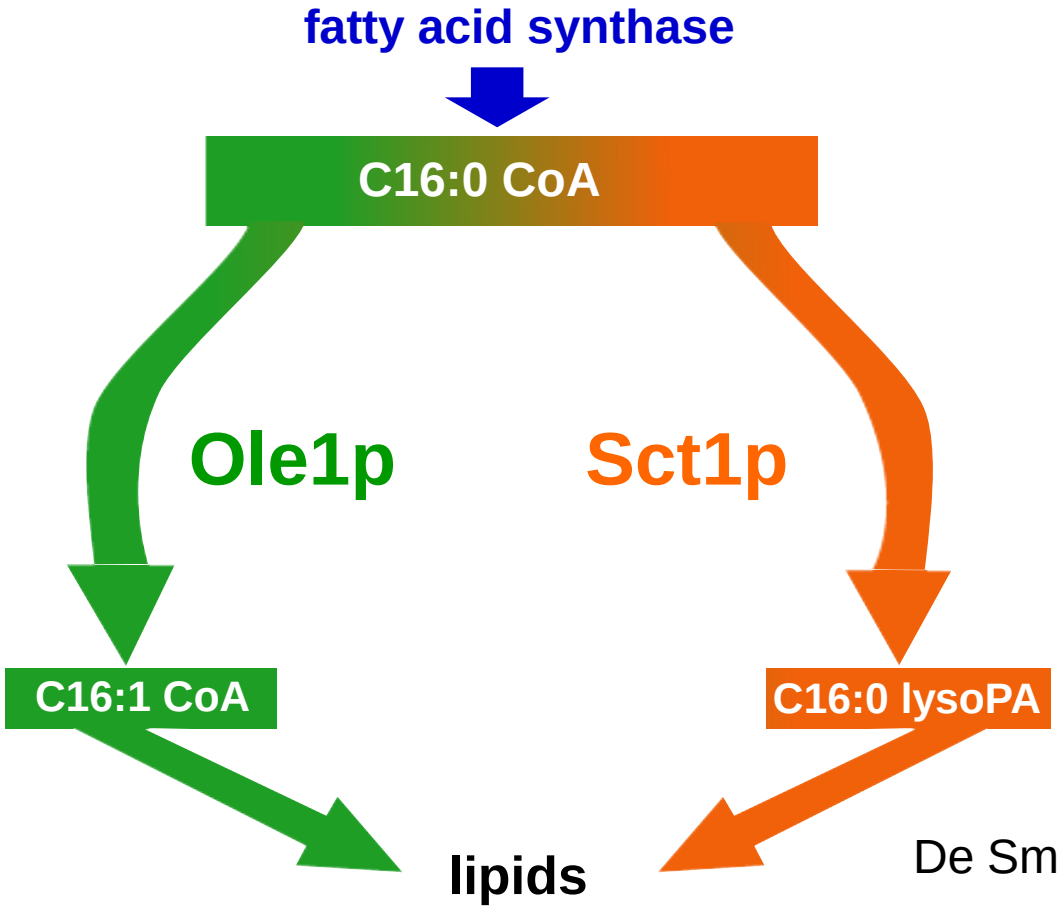


Gas chromatography analysis of fatty acid methyl esters from total lipid extracts (n = 2)



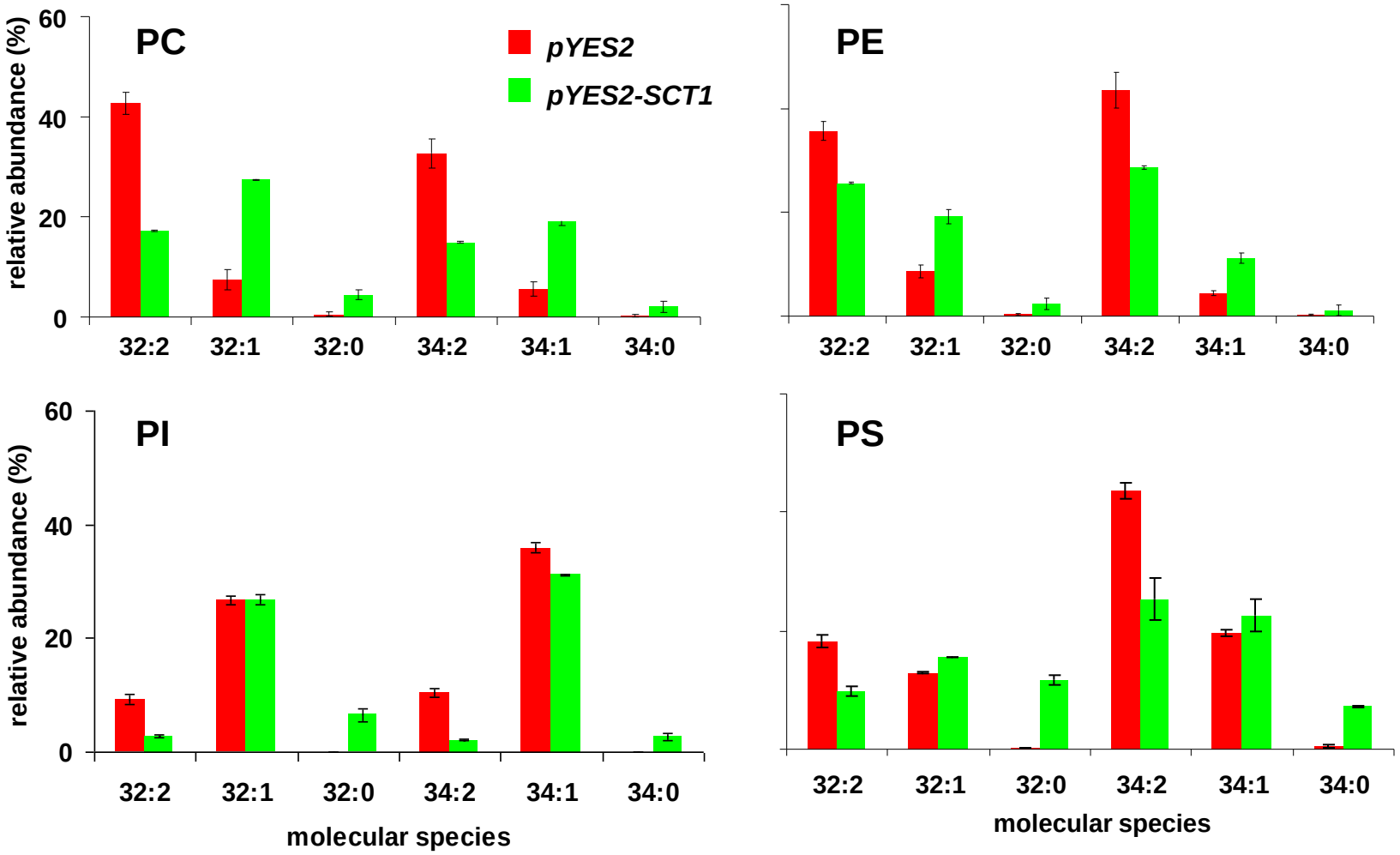
Sct1p regulates acyl chain desaturation by competing for C16:0 CoA with the desaturase Ole1p

- Deletion decreases C16:0 by 50%
- Overexpression strongly increases fatty acid saturation



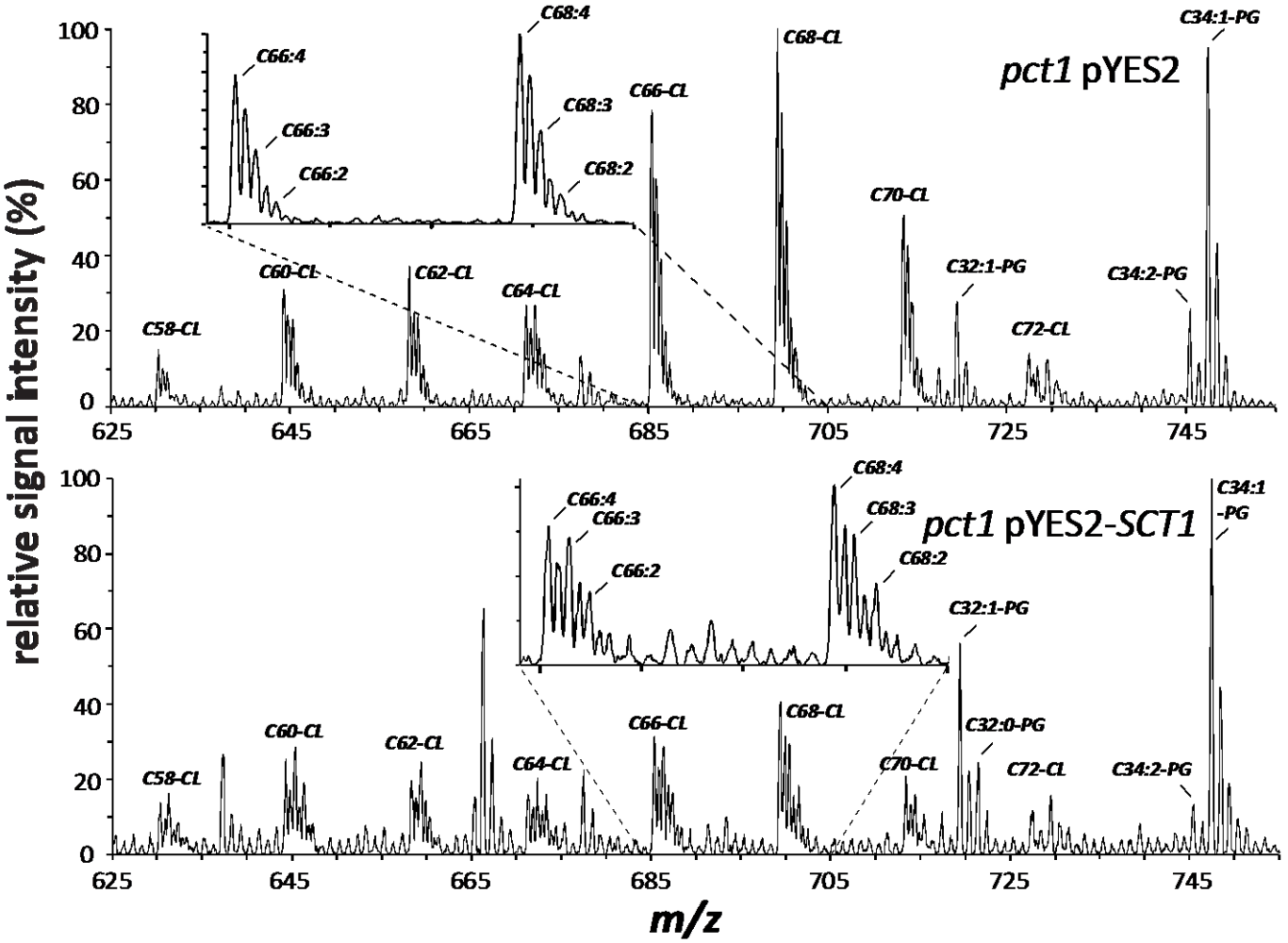
De Smet et al., MBoC,2012

Overexpression of Sct1p: increased saturation in molecular species profiles of the major phospholipids:

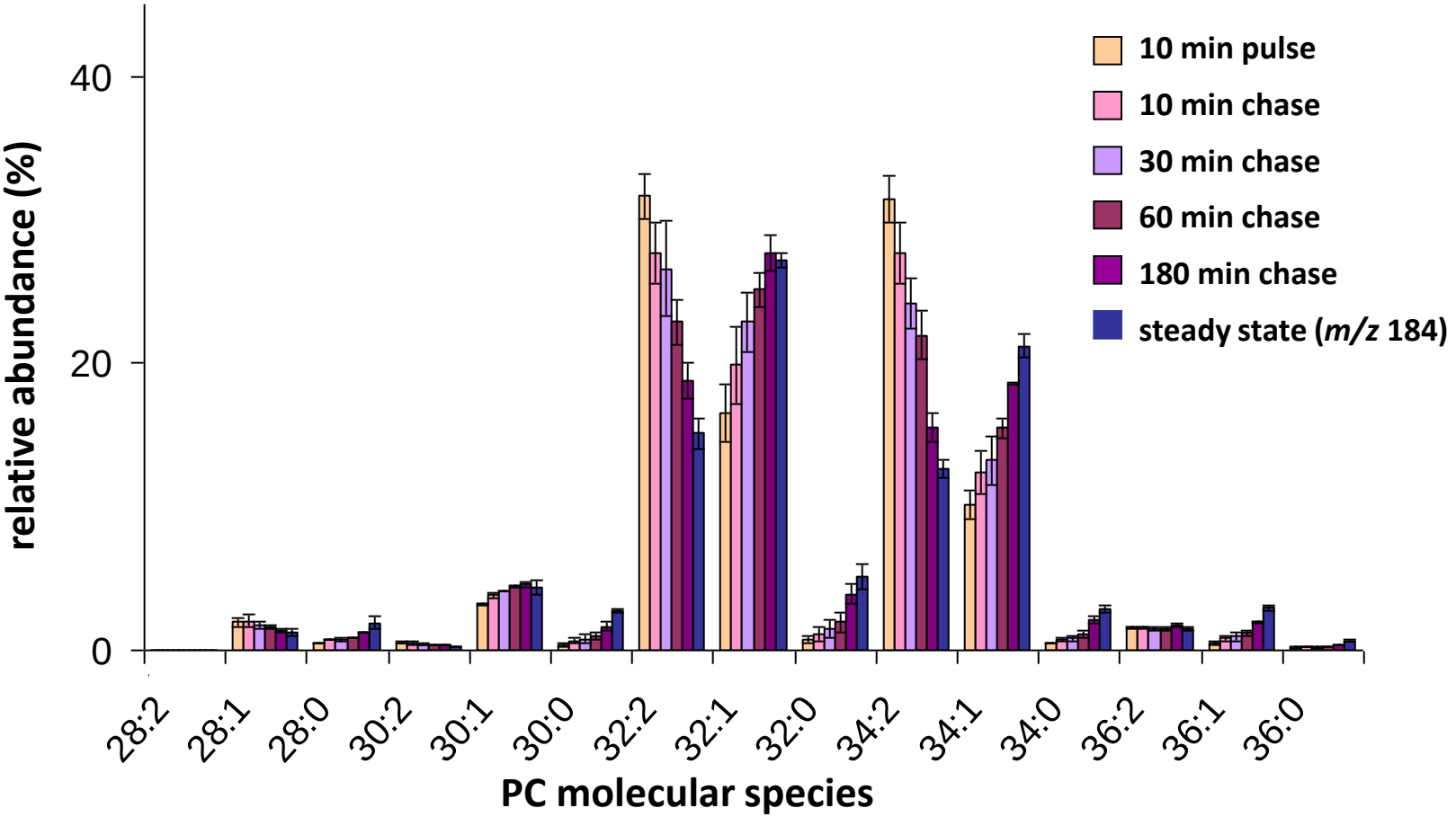


Total lipid extracts analyzed by ESI-MS/MS (n=2)

Effect of overexpressing Sct1p on molecular species profile of CL

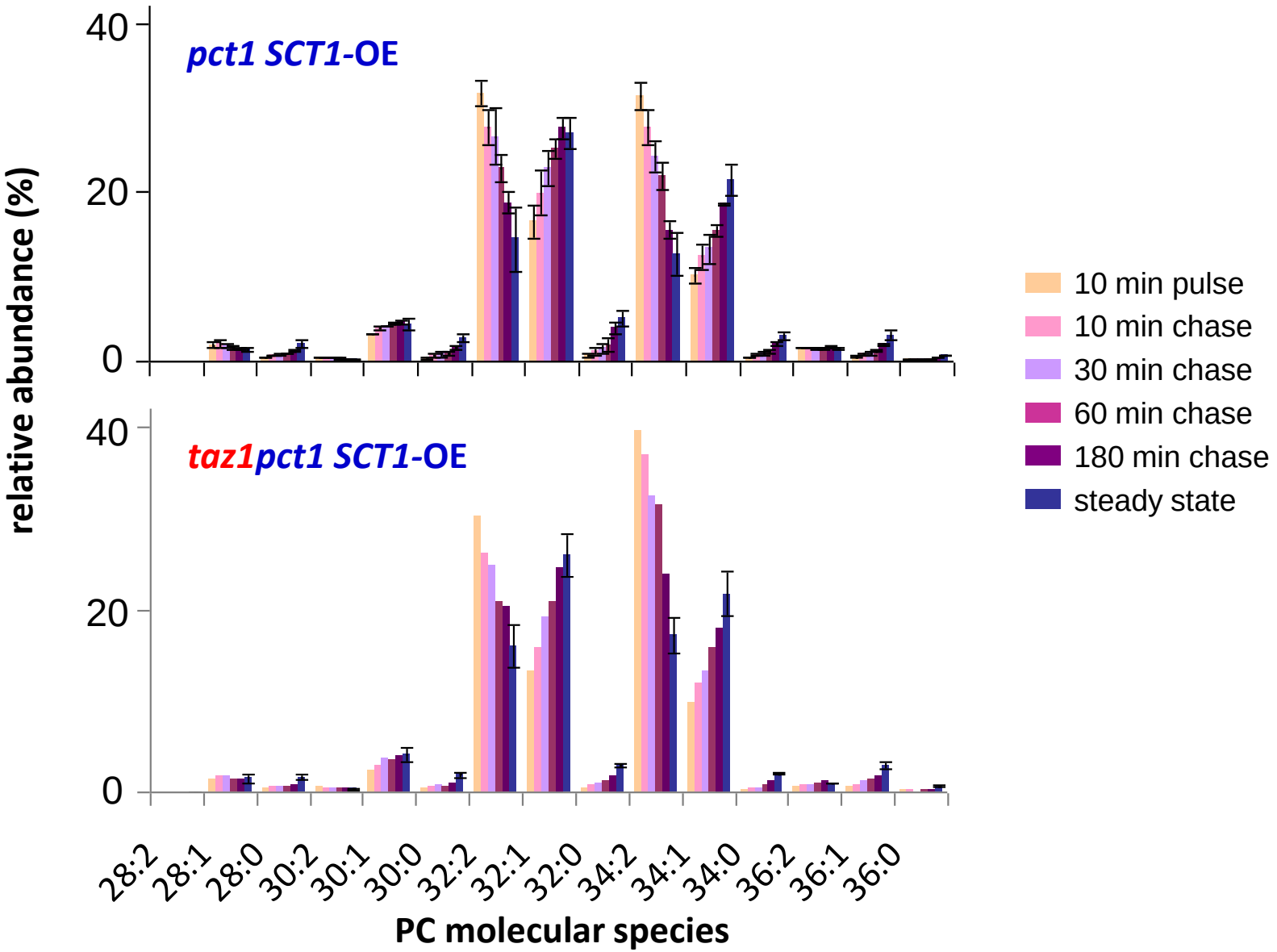


SCT1 overexpression enhances the extent of PC remodeling, and is a new tool in screening for genes involved

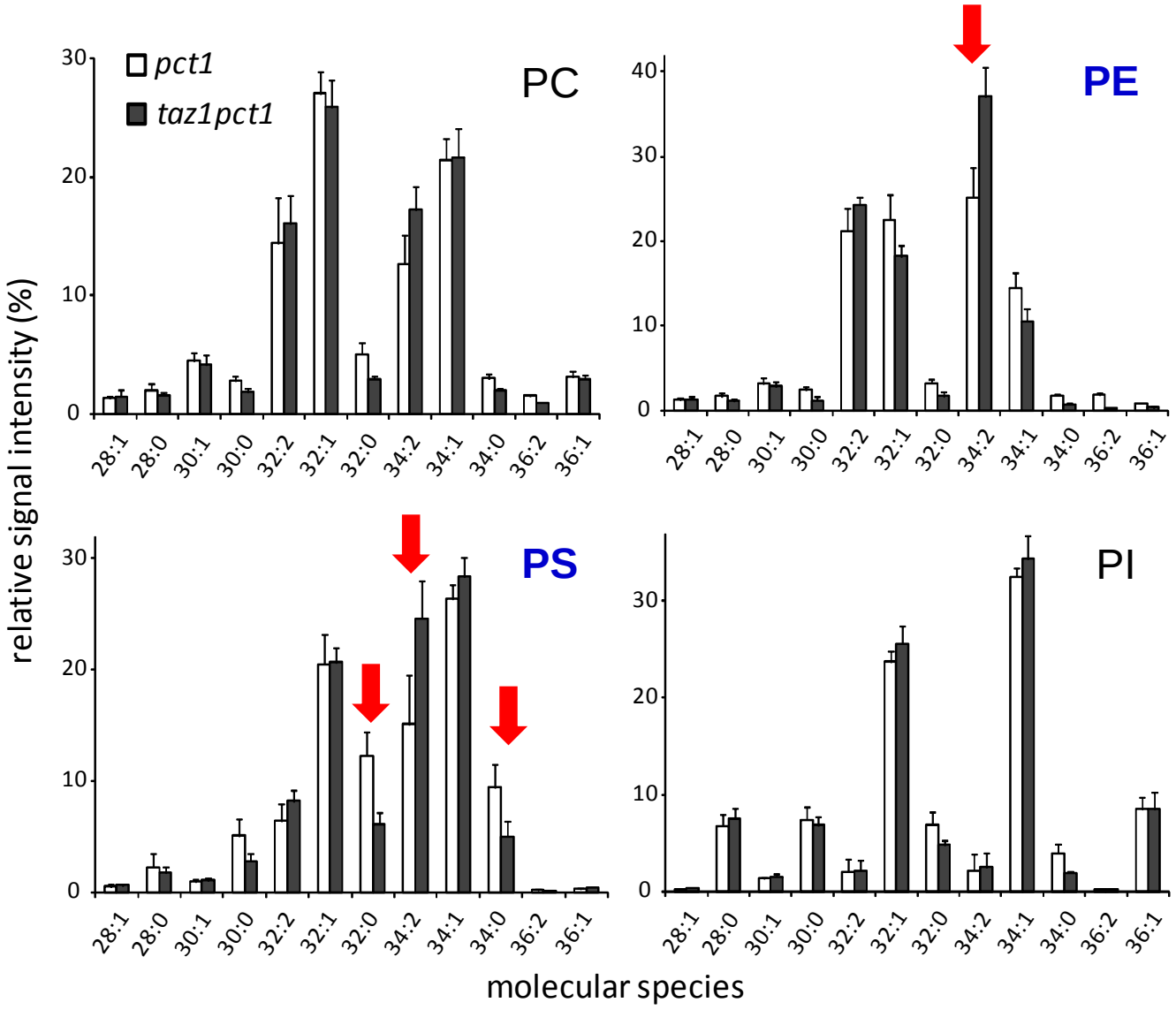


***pct1* pYES2-SCT1 cells** were pulsed for 10 min with **(*methyl*-D_3)-methionine**, the label was chased with (*methyl*-H_3)-methionine

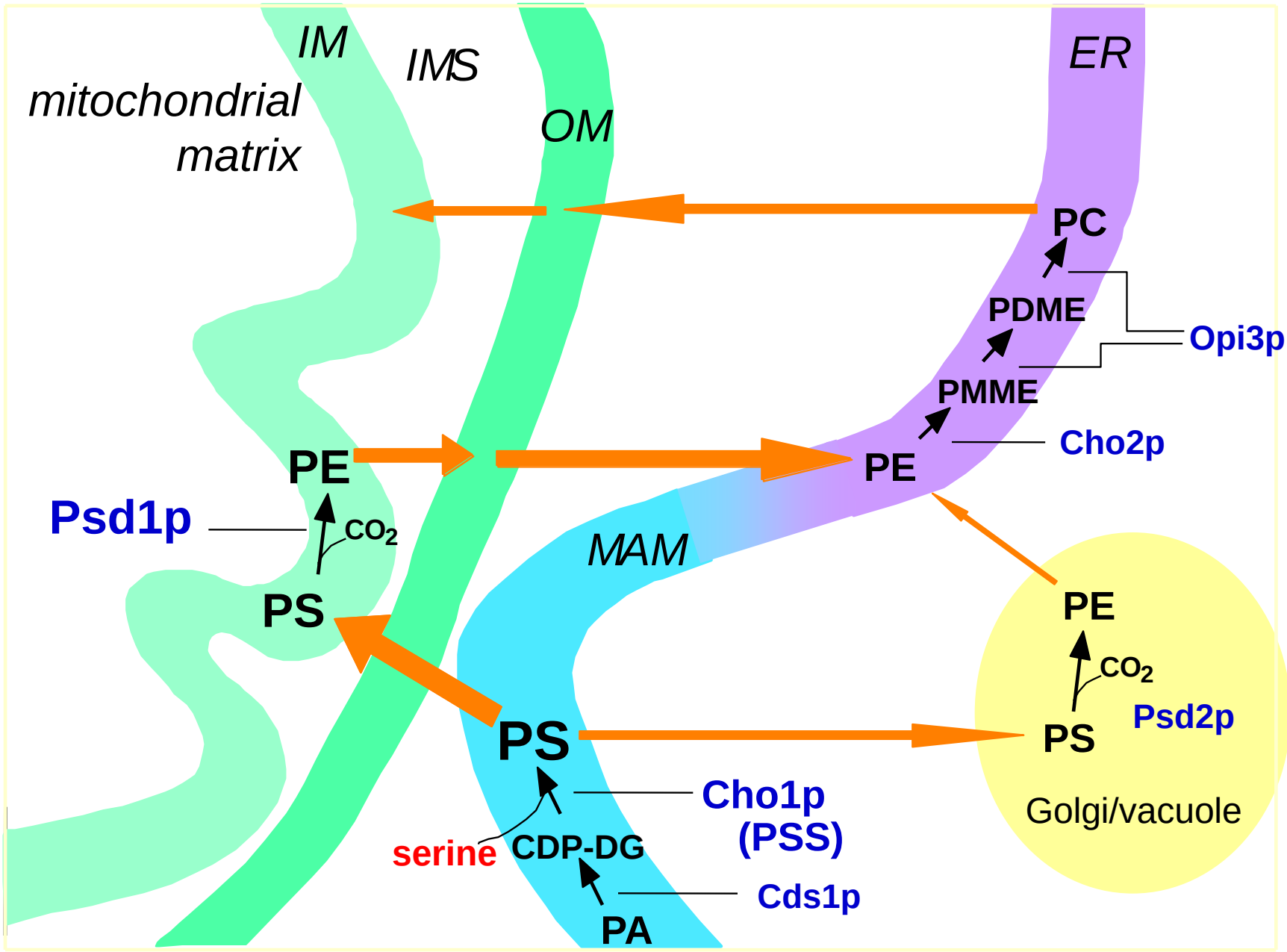
Deletion of *TAZ1* does not affect remodeling of PC in the *SCT1*-overexpression background



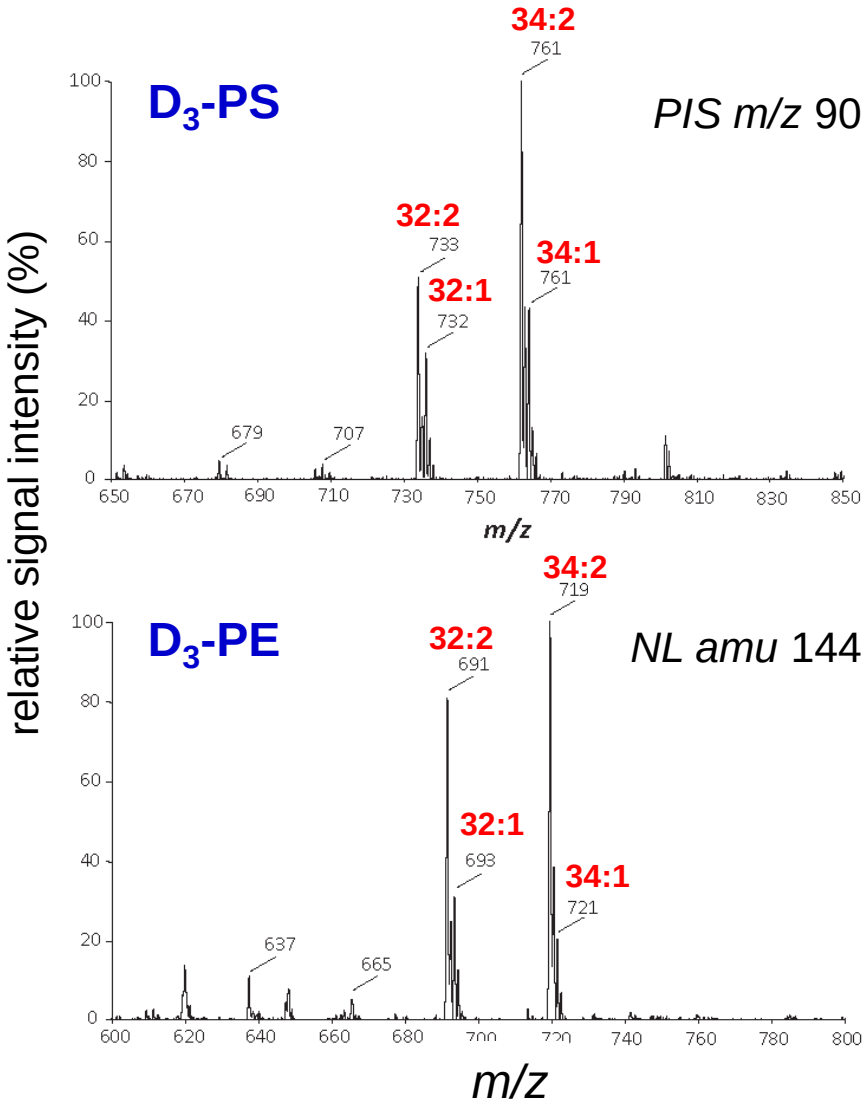
Deletion of *TAZ1* increases the level of unsaturation of PS and PE in the *SCT1*-overexpression background



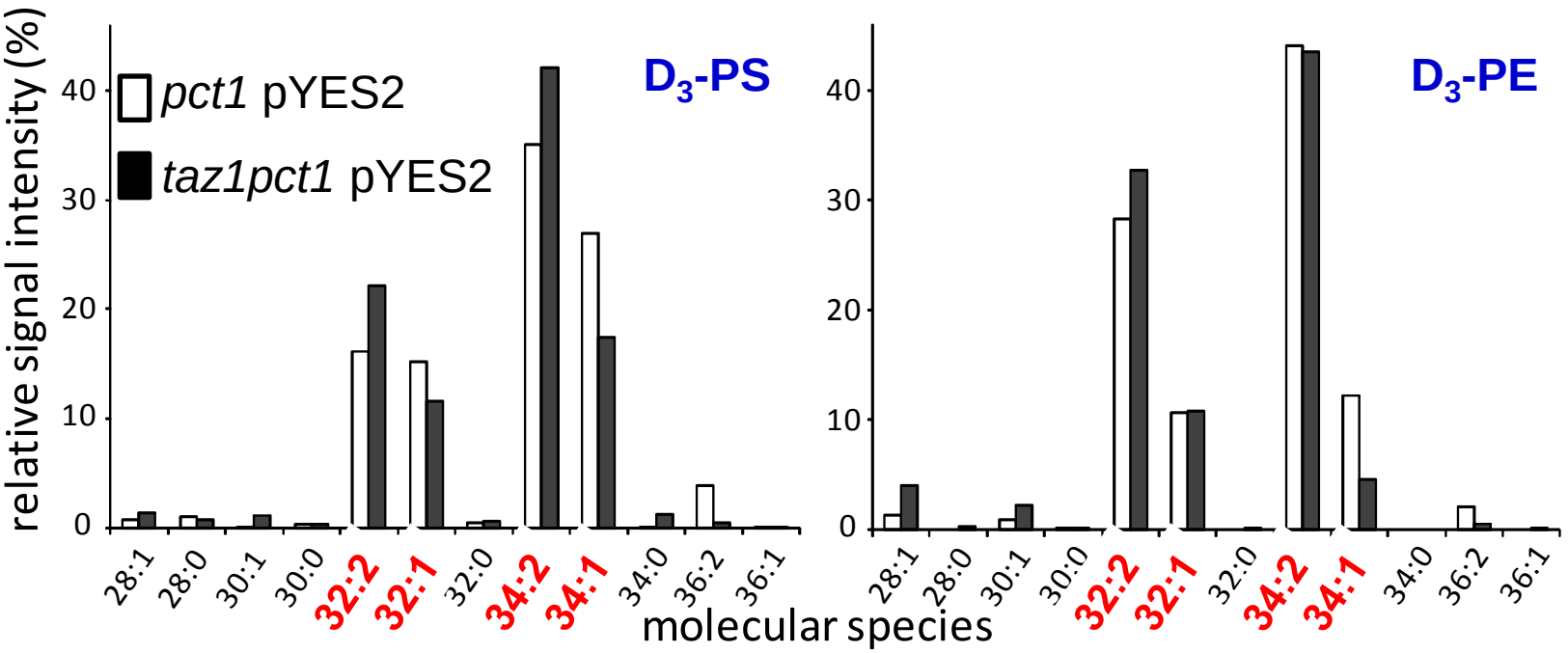
PS in yeast aminophospholipid metabolism



Pulse labeling for 20 min with $^{2}H_3$-serine reveals newly synthesized PS and PE

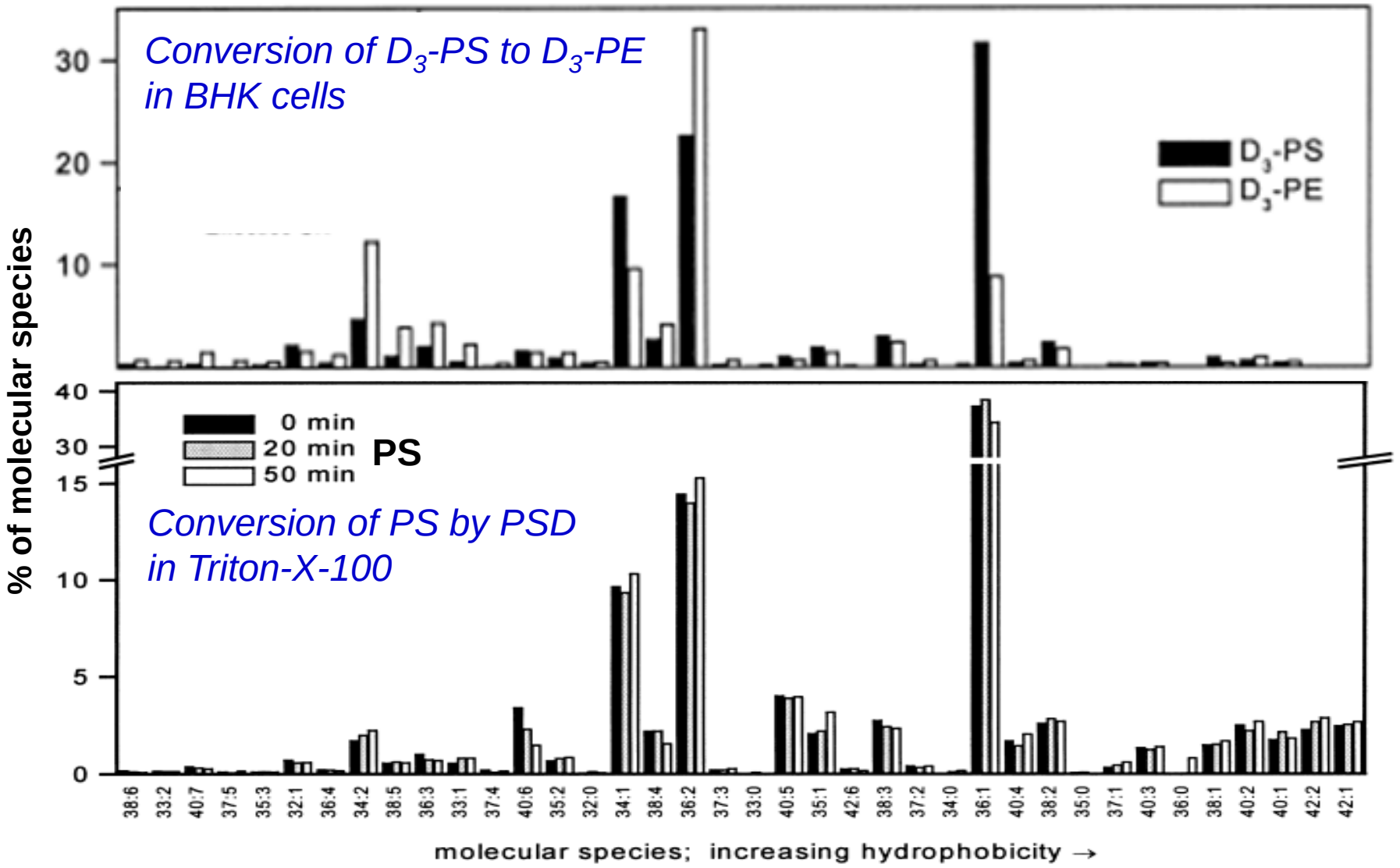


Species selectivity of decarboxylation by PS decarboxylase:
32:2 > 34:2 > 32:1 > 34:1



cells were pulsed for 20 min with **D_3-serine** before lipid extraction

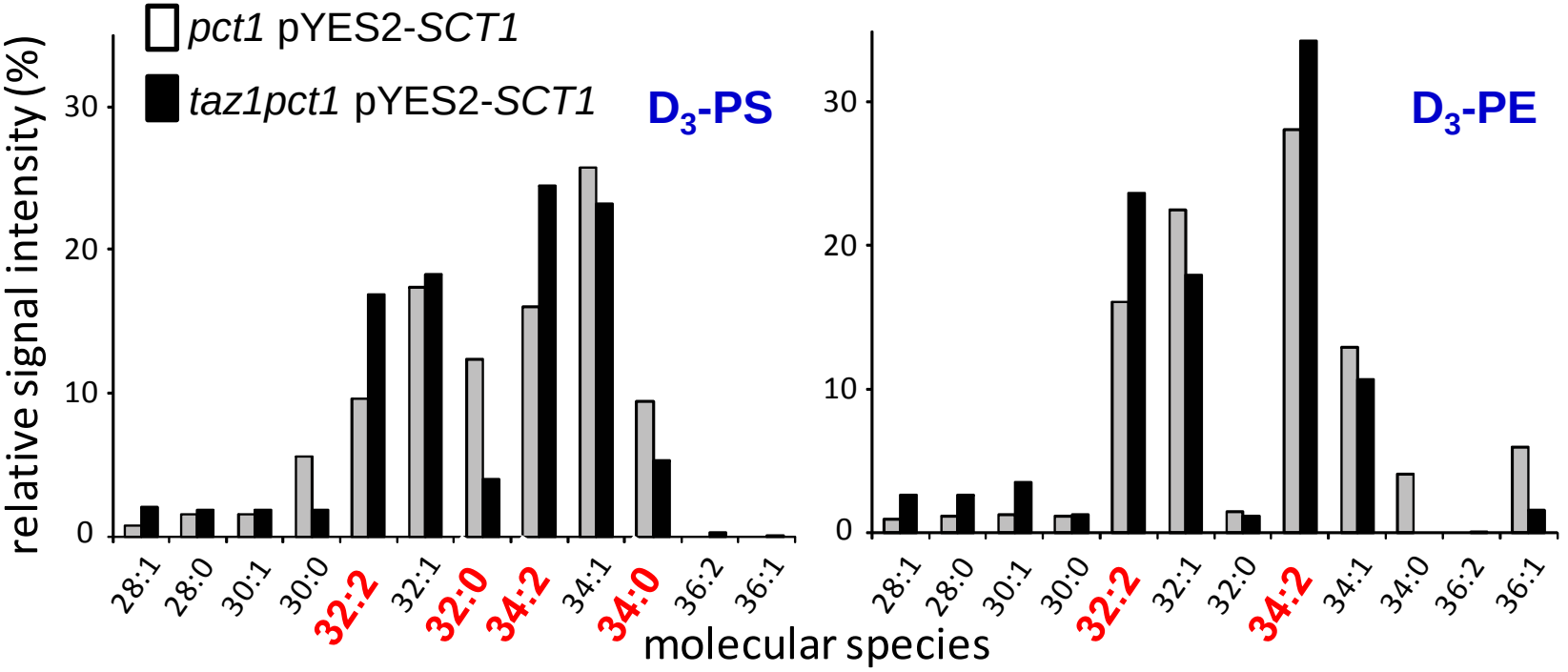
Translocation of PS to mitochondria diminishes with increasing molecular hydrophobicity



taken from Heikinheimo & Somerharju (2002) Traffic 3: 367

Overexpression of *SCT1*:

Deletion of *TAZ1* reduces the *relative* content of saturated acyl chains in newly synthesized D_3-PS and to a lesser extent in D_3-PE



cells were pulsed for 20 min with **D_3-serine** before lipid extraction

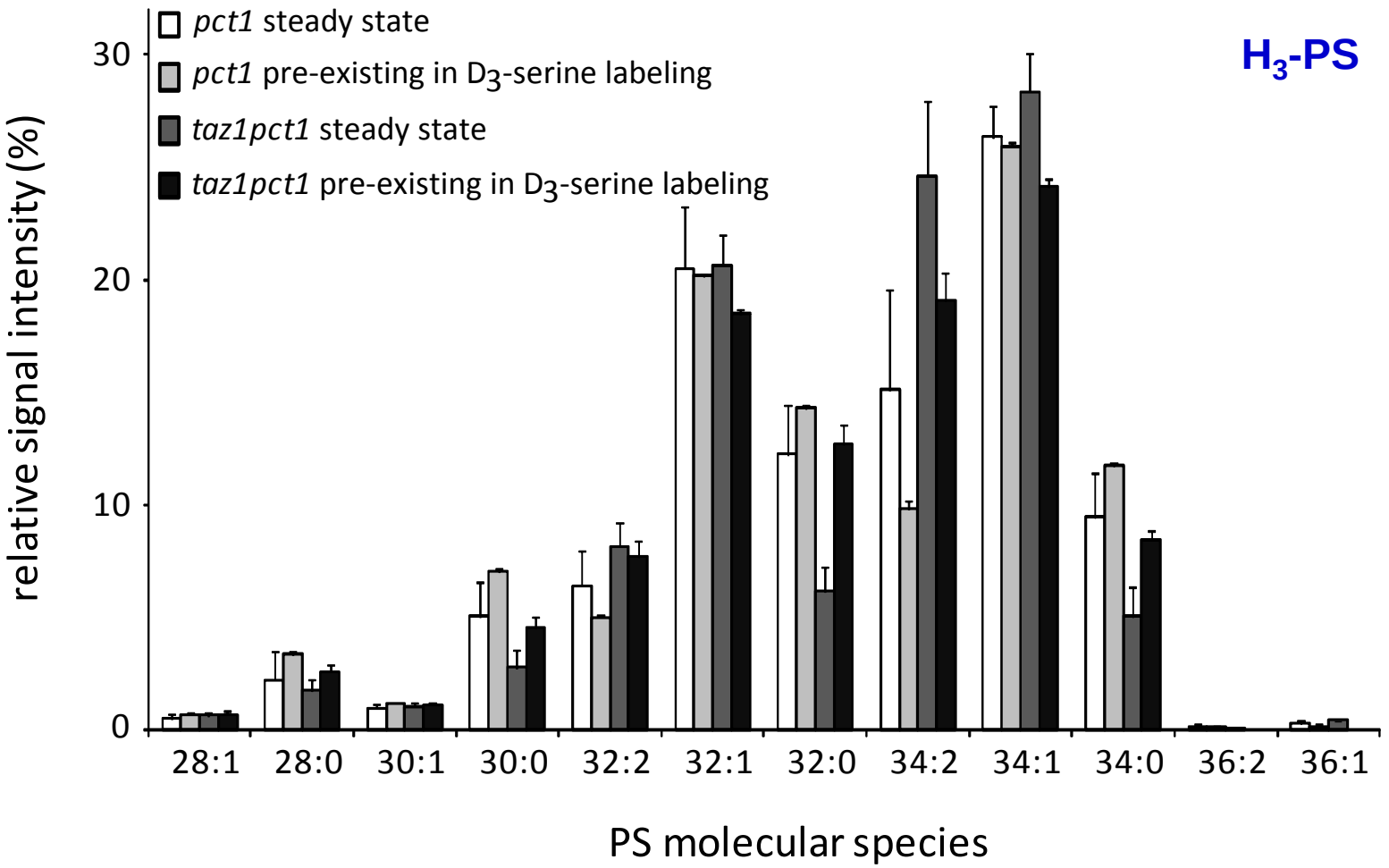
In the absence of Taz1p, the relative amount of ^{32}P-PS is doubled after a 15 min pulse with $^{32}P_i$*

Strain	^{32}P in phospholipid (% of total)						
	CL	PA	PE	PS	PI	PC	
wt	1.38	15.90	5.47	**12.36**	52.03	5.59	*log phase*
taz1	2.19	8.71	5.79	**23.14**	50.20	6.62	
wt	1.38	16.86	3.25	**10.01**	57.45	6.85	*early stationary*
taz1	2.65	15.03	2.84	**20.04**	49.75	7.32	

W303-1A (wt) and isogenic BTY1 (*taz1Δ*) were cultured on YPGE

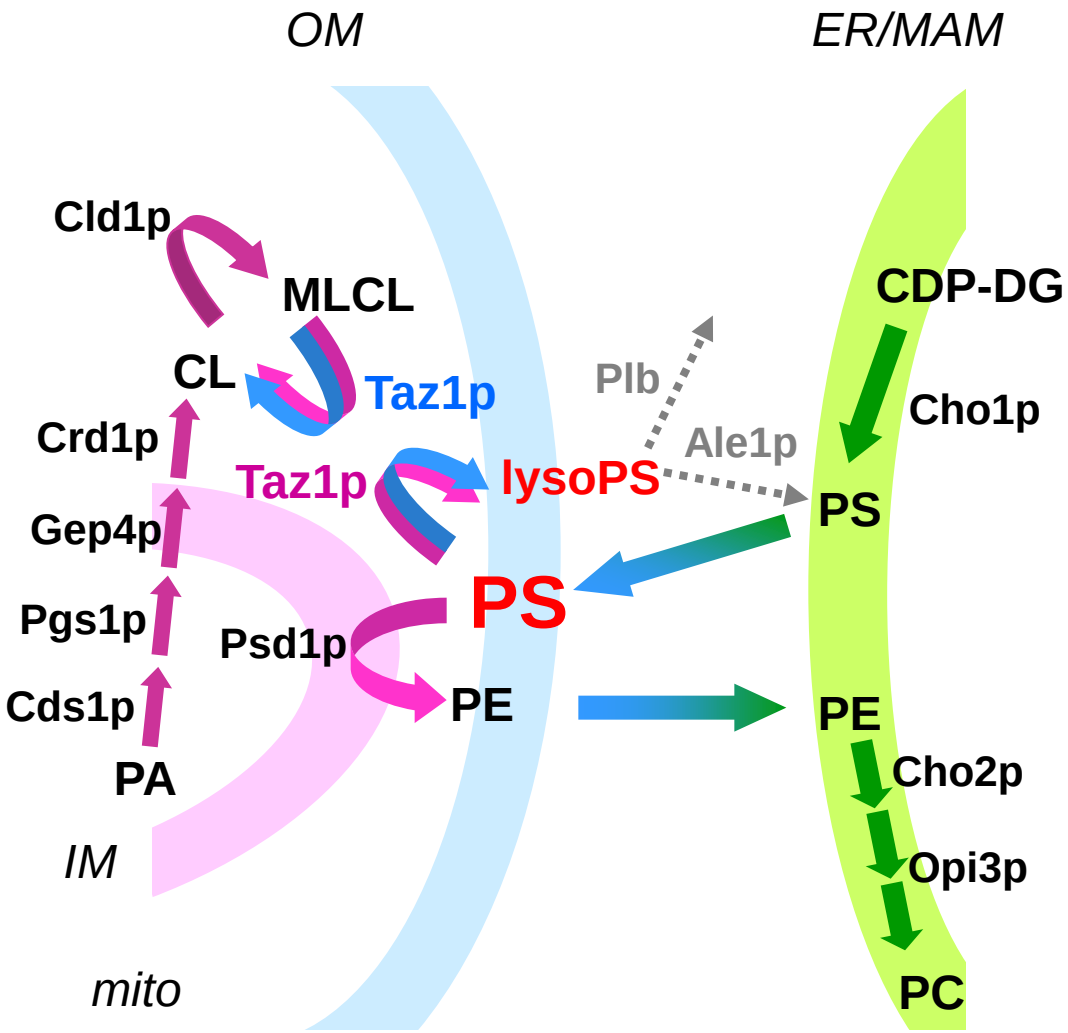
*taken from Gu *et al.* (2003) Mol. Microbiol. 51: 149

In the absence of Taz1p the maturation of the PS molecular species profile is delayed in *SCT1*-overexpressing cells



Comparison of species profiles of ***steady state*** PS to ***pre-existing*** ^{1}H-PS pool in ^{2}H-serine labeling

Taz1p uses PS as acyl chain donor



Conclusions and implications

- Overexpression of *SCT1* diversifies the molecular species profile by increasing the content of C16:0, making it a useful tool in dynamic lipidomics studies in yeast

- The decarboxylation of PS to PE decreases with increasing hydrophobicity of the PS substrate in yeast (as in BHK cells)

- The effects of deleting *TAZ1* on the species profiles of (newly synthesized) PS indicate that Taz1p consumes PS; other acyl chain donors are not excluded

➢ ***The localization of Taz1p in the MOM allows it to compete with Psd1p for incoming PS***

➢ ***Using incoming PS as preferred acyl donor would increase the efficiency of Taz1p (no acyl chain specificity!) in enriching CL with unsaturated acyl chains***

Acknowledgments

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