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REVIEW

Three, two, one yeast fatty acid desaturases: regulation and function

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Abstract Fatty acid composition of biological membranes functionally adapts to environmental conditions by changing its composition through the activity of lipid biosynthetic enzymes, including the fatty acid desaturases. Three major desaturases are present in yeasts, responsible for the generation of double bonds in position C9–C10, C12–C13 and C15–C16 of the carbon backbone. In this review, we will report data addressed to define the functional role of basidiomycete and ascomycete yeast desaturase enzymes in response to various external signals and the regulation of the expression of their corresponding genes. Many yeast species have the complete set of three desaturases; however, only the Δ9 desaturase seems to be necessary and sufficient to ensure yeast viability. The evolutionary issue of this observation will be discussed.

Keywords Lipid biosynthesis · Unsaturated fatty acids · Membranes · Evolution

Introduction

Fatty acids (FAs) are a family of molecules characterized by the length of the carbon backbone and by the presence or absence, number and position of carbon–carbon double bonds. Commonly found FAs range from 16 to 24 carbon atoms and have from 1 to 6 double bonds, when present. Shorter and longer FAs are also found in lower amounts. FAs are essential components of cytoplasmic membranes and determine structural and functional properties of the membranes on the basis of their assortment in the phospholipidic moiety. Unsaturated FAs (UFAs) contain one (Monounsaturated FAs: MUFAs) or many (Polyunsaturated FAs: PUFAs) double bonds and their presence in the phospholipids allows to adapt membrane fluidity in response to changing conditions. FAs are also carbon and energy reservoirs and participate in additional cellular functions; for example, protein modification and biosynthesis of other metabolites, like sphingolipids and ceramides, prostaglandins and leukotrienes.

Steps of *de novo* FAs biosynthesis are strongly conserved among organisms and require acetyl-CoA, carbon dioxide and NADPH. In yeasts, the reaction is initiated by the Acetyl-CoA Carboxylase Acc1 that produces malonyl-CoA. Polymerization is then obtained by cyclic addition of acyl groups to malonyl-CoA and reduction to methylene of the resulting β-carbonyl bond. These steps are carried out by the Fatty Acid Synthases Fas1 and Fas2, which are the β and α subunits, respectively. Fas2 contains the Acyl Carrier Protein (ACP) domain (Mohamed et al. 1988). Polymerization produces palmitic (C16:0) and stearic (C18:0) acids (Fig. 1). Longer FAs, up to C24-C26, can be synthesized in the yeast *Saccharomyces cerevisiae* by the Elongases Elo2/Fen1 and Elo3 (Oh et al. 1997). The family of UFAs is then generated, starting from the C16:0 and C18:0 substrates, by the action of Fatty Acid Desaturases (FADs). FADs are redox enzymes that introduce carbon–carbon double bonds using molecular oxygen as electron acceptor, and the saturated FAs and cytochrome b5 or Ferredoxin as electron donors. Oxygen is activated by coordination with the iron atoms bound to the histidine rich sequences present

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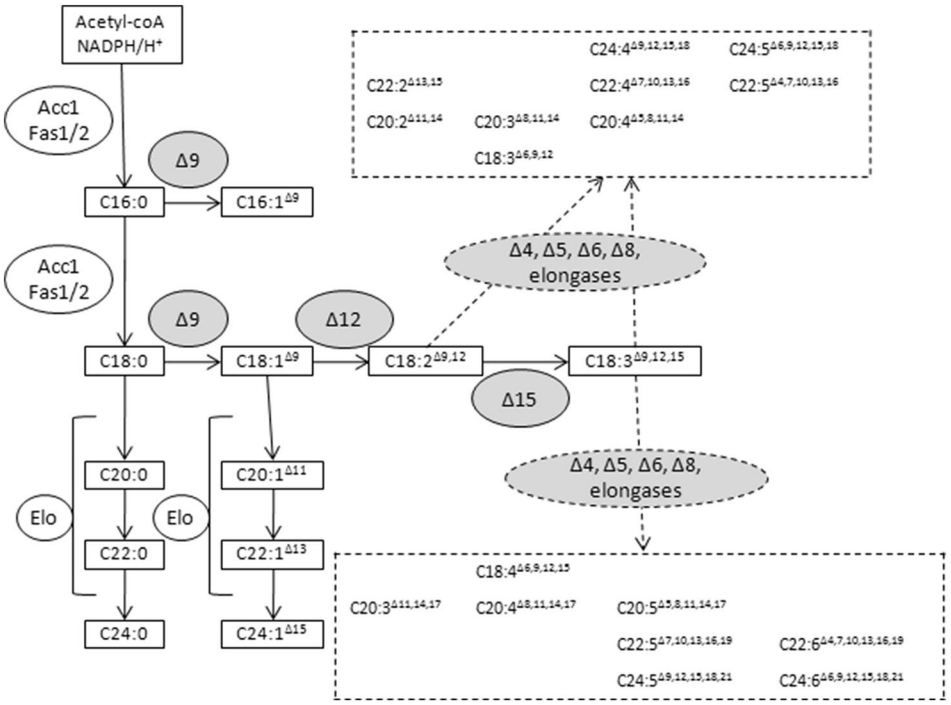

Fig. 1 Scheme of Fatty Acids biosynthesis. Metabolites are reported in *squared boxes*; enzymes are reported in ovals. Vertical steps correspond to elongation of the carbon backbone, horizontal steps correspond to desaturation reactions. *Dotted lines* include non-yeast biosynthetic steps and metabolites/enzymes

in the FAD enzymes (Martin et al. 2007). Cytochrome b5 (Cytb5) is regenerated by a NADH-dependent Cytb5 Dehydrogenase while Ferredoxin is recycled by Ferredoxin Oxidoreductases.

FADs can be classified by cellular localization, by the FA substrate and by the position of the double bond they introduce (Los and Murata 1998). In yeasts, FADs are ER bound enzymes and use Acyl-CoA as substrate and Cytochrome b5 as electron donor. Depending on double bond position, FA desaturases can be divided into Stearoyl-CoA Desaturases (SCDs), Omega Desaturases and Front-End Desaturases (Hashimoto et al. 2008): SCDs introduce a double bond at position 9 (Δ9 Desaturases) of palmitic (C16:0) or stearic (C18:0) acids and generate palmitoleic and oleic acids (C16:1$^{\Delta 9}$ and C18:1$^{\Delta 9}$). These enzymes are present in all eukaryotes examined so far. The Omega Desaturases introduce a double bond between the alkyl terminus of the FA and an already existing double bond. They synthesize C18:2$^{\Delta 9,12}$ (linoleic acid) and C18:3$^{\Delta 9,12,15}$ (α-linolenic acid, ALA) and are also called Δ12 and Δ15 Desaturases, respectively. The Front-End group of desaturases introduce double bonds between the carboxylic terminus of the FA and a preexisting double bond and are named Δ4, Δ5, Δ6 and Δ8 Desaturases. Another group of desaturases is the Δ4 Sphingolipid Desaturase, specifically involved in sphingolipid biosynthesis. The FA biosynthetic network is completed by elongases activities. Two families of elongases can be distinguished: elongases active on saturated FAs or on MUFAs (S/MUFA Elongases) and elongases active on PUFAs. S/MUFA Elongases are widely distributed and have substrate length specificity, like the yeast Elongases Elo1, Elo2 and Elo3 (Toke and Martin 1996; Oh et al. 1997). PUFA Elongases are absent in fungi and plants (Hashimoto et al. 2008).

The structure of ER-bound FAD enzymes is characterized by three typical histidine rich sequences, spaced by trans-membrane (TM) domains, essential to hold iron atoms in the catalytic center on the cytoplasmic side of the membrane. A large scale analysis of eukaryotic desaturases (Hashimoto et al. 2008) revealed that the precise sequence of the histidine rich domains is specific for the already cited SCD, Omega, Front-End and Sphingolipid Desaturases. In fungi, the SCDs have a Cyt b5 domain in the carboxy terminal part of the enzyme. Differently, the Omega Desaturases lack the Cyt b5 domain, indicating that desaturases might also react with free Cytochrome b5 to exchange reducing power. Evolutionary studies showed that, among Omega Desaturases, the Δ12 enzymes are ancestors of the Δ15 subfamily (Wang et al. 2013). Interestingly, fungal Omega Desaturases have been described to have bifunctional Δ12/Δ15 activities (Buček et al. 2014; Cui et al. 2016). Among the Front-End Desaturases, only the widely diffused Δ5 and Δ6 Desaturases have been found also in fungi (Michaelson et al. 1998; Sakurdani et al. 1999; Laoteng et al. 2000; Hong et al. 2002a, b). Differently from SCDs, these desaturases contain the Cyt b5 domain in the N-termini of the proteins.

An increasing interest is arising around yeast and, in general, fungal desaturases due to potential biotechnological application and health issues deriving from the

important regulatory functions of PUFAs in human physiology. Engineered yeasts and fungi can be developed to increase the oil content in oleaginous species for biofuel production (see for example Qiao et al. 2015; Wang et al. 2016), for production of industrially important FAs (Meesapyodsuk et al. 2015) or PUFAs with nutritional value (Li et al. 2009; Kimura et al. 2009; Wan et al. 2009).

Yeast fatty acid desaturases

To date, literature indicates that yeasts have one, two or three different desaturases belonging to the SCD and Omega (Δ12 and Δ15) Desaturase families. In order to investigate the distribution of these enzymes among yeast species, we used the yeast *Kluyveromyces lactis*, which has been demonstrated to harbor all three desaturases (Kainou et al. 2006; Micolonghi et al. 2012), to explore the large yeast sequence database of Genome Resources for Yeast Chromosomes (GRYC: http://gryc.inra.fr/index.php?page=home) containing the annotated full sequences of 34 yeast species. The proteins used as query were KlOle1, Fad2 and Fad3, encoded by genes KLLA0C05566g, KLLA0F07095g and KLLA0B00473g, respectively. Protein sequences were aligned to the database sequences using the BLASTP (2.4.0+) algorithm. Results are summarized in Table 1.

SCD (Δ9) desaturases. All the yeast species contained a gene coding for SCD (Table 1), suggesting that this enzyme is essential for cell viability and ensures a minimal qualitative (only MUFAs) desaturation to membranes for proper functionality. Comparative analysis of the protein sequences revealed a high identity (59.7–82.6%) distributed along the whole protein (Table 2) except for *Lachancea waltii* SCD, that was 84.4% identical to KlOle1 but only in 339 amino acids of the C-terminus portion, and for SCDs of *Arxula adeninivorans* and *Yarrowia lipolytica* that showed identity (48.3–57.8%) in 425 and 408 amino acids, respectively. *A. adeninivorans* and *Y. lipolytica* are also evolutionary more distant yeasts. SCD with limited identity were found also in *Cyberlindnera fabianii* (CYFA0S07e03004g) and *Kuraishia capsulata* (KUCA_T00001427001). Yeasts of the *Pichia/Hansenula* group, of the *Candida* group and *K. capsulata* had two or more genes coding for SCDs. *Pichia/Hansenula* and *Candida* yeasts belong to the CTG clade, that is characterized by a change in the genetic codon CUG from leucine to serine (Butler et al. 2009), and the presence of two SCD genes in *Candida* and *Pichia* has been demonstrated by previous studies (Krishnamurthy et al. 2004; Yu et al. 2012b). Duplication of SCD genes in the clade suggests that this event occurred early in the evolution of this branch. The finding of duplicated SCD genes also in *K. capsulata*, that diverged before the CTG event (Morales et al. 2013), might help to locate the gene duplication upstream to clade divergence. Gene redundancy was even more pronounced in *Millerozyma farinosa*, in which four copies of SCD genes were present: genes PISO0I17894g and PISO0J19655g shared about 95% identity and genes PISO0B11617g and PISO0A11550g were 100% identical. A preliminary synteny analysis showed that proximal genes were identical for the homologous genes, but different between pairs, indicating independent duplication events. This finding is coherent with the polyploidization event described for this yeast (Mallet et al. 2012).

Omega (Δ12) *desaturases. K. lactis* Δ12 (Fad2) and Δ15 (Fad3) desaturases share 62.4% identity in 386 amino acids (Fad2 and Fad3 are 410 and 415 amino acids long, respectively): the specificity of their activity has been established by the determination of their biosynthetic products in the heterologous yeast *S. cerevisiae* (Kainou et al. 2006) and in *K. lactis* deleted strains (De Angelis et al. 2016). Due to the high similarity of the two proteins, discrimination between Δ12 and Δ15 enzymes by simple sequence alignment might not be conclusive. Indeed, the alignment of the GRYC database with *K. lactis* Fad2 and Fad3 yielded two overlapping sets of proteins with very high identity. However, a block of sequences with best scores to Fad2 was exactly the block with lower scores to Fad3 and *viceversa*. In addition, any yeast species was represented only once in each block. These observations allowed us to discriminate between Δ12 and Δ15 enzymes in each yeast species, although a confirmation by assaying the actual activity might be necessary.

Δ12 desaturases had 56.8–73.9% identity values (Table 2) and were found in all yeast species (Table 1), except those belonging to the WGD (Whole Genome Duplication) clade. The WGD allowed the occurrence in the following evolution of different events, including multiplication of some genes like glycolytic genes and sugar transporter genes, and loss of some metabolic pathways (Piškur and Langkjær 2004). The absence of desaturases for the synthesis of PUFAs in this clade might be the consequence of such loss events. In these yeasts, the modulation of membrane properties and functionality, for example fluidity, can be thus accomplished only by varying the relative amount of MUFAs or the FA backbone length and probably reflects the adaptation to environments subjected to reduced variability. All Δ12 desaturases were encoded by single genes except in *M. farinosa* that, similarly to the previously described SCDs, had two Δ12 genes (PISO0C09088g and PISO0D09155g) sharing 100% identity and with identical genomic environs but placed on different chromosomes. Δ12 desaturases of *A. adeninivorans, Y. lipolytica, K. capsulata* and *M. farinosa* showed lower identity values to Fad2 than the other desaturases (56.8–58.6%,

Table 1 Yeast fatty acid desaturase genes

Clade	Group	Yeast name and strain	Δ9 Desaturase	Δ12 Desaturase	Δ15 Desaturase
		Arxula adeninivorans LS3	ARAD1D01914g	ARAD1D11396g	
	Yarrowia	*Yarrowia lipolytica* A101	YALIA101S01e24058g	YALIA101S01e13674g	
		Yarrowia lipolytica CLIB122	YALI0C05951g	YALI0B10153g	
		Kuraishia capsulata CBS 1993	KUCA_T00002566001 KUCA_T00001427001	KUCA_T00005823001	
CTG	Pichia, Hansenula	*Cyberlindnera fabianii* YJS4271	CYFA0S04e03422g CYFA0S10e01838g CYFA0S07e03004g	CYFA0S32e00958g	CYFA0S01e00826g
		Debaryomyces hansenii CBS 767	DEHA2F03872g DEHA2F24002g	DEHA2G05346g	DEHA2E14542g
		Millerozyma farinosa CBS 7064	PISO0I17894g/ PISO0J19655g PISO0B11617g/ PISO0A11550g	PISO0C09088g/ PISO0D09155g	
	Candida	*Candida albicans* SC5314	C1_08360C_A C2_07090C_A	C6_01110W_A	C1_13070C_A
		Candida parapsilosis CDC 317	CPAR2_206900 CPAR2_406570	CPAR2_603730	CPAR2_801860
KLE	Lachancea	*Lachancea cidri* CBS 2950	LACI0B05468g	LACI0G04676g	LACI0G19350g
		Lachancea dasiensis CBS 10,888	LADA0A03994g	LADA0E11870g	LADA0A03312g
		Lachancea fantastica CBS 6924	LAFA0C04676g	LAFA0D09868g	LAFA0C03928g
		Lachancea fermentati CBS 6772	LAFE0C07360g	LAFE0E07140g	LAFE0C06612g
		Lachancea kluyveri CBS 3082	SAKL0A07326g	SAKL0H03872g	SAKL0A06556g
		Lachancea lanzarotensis CBS 12,615	LALA0S07e00716g	LALA0S01e11056g	LALA0S13e03730g
		Lachancea meyersii CBS 8951	LAME0C06876g	LAME0F06942g	LAME0C07558g
		Lachancea mirantina CBS 11,717	LAMI0G09076g	LAMI0A03136g	LAMI0F08526g
		Lachancea nothofagi CBS 11,611	LANO0F02872g	LANO0H18074g	LANO0F02212g
		Lachancea quebecensis CBS 14,088	LAQU0S02e09736g	LAQU0S01e03378g	LAQU0S16e00650g
		Lachancea thermotolerans CBS 6340	KLTH0H06798g	KLTH0D13640g	KLTH0E10912g
		Lachancea waltii CBS 6430	LAWA0H05556g	LAWA0E13432g	LAWA0H04896g
		Kluyveromyces lactis CBS 2359	***KLLA0C05566g***	***KLLA0F07095g***	***KLLA0B00473g***
		Eremothecium gossypii ATCC 10,895	AGOS_AAR153C	AGOS_AFR589C	
ZT	Zygosaccharomyces	*Zygosaccharomyces bailii* CBS 680	ZYBA0S02-13410g	ZYBA0S07-01530g	
		Zygosaccharomyces rouxii CBS 732	ZYRO0C05016g	ZYRO0B07414g	

Table 1 (continued)

Clade	Group	Yeast name and strain	Δ9 Desaturase	Δ12 Desaturase	Δ15 Desaturase
WGD		*Tetrapisispora blattae* CBS 6284	TBLA_0C06220		
		Naumovozyma castellii CBS 4309	NCAS_0D03250		
		Kazachstania naganishii CBS 8797	KNAG_0F03250		
	Nakaseomyces	*Nakaseomyces bacillisporus* CBS 7720	BN123_NABA0s37e00418g		
		Nakaseomyces castellii CBS 4332	BN121_CACA0s33e03014g		
	Nakaseomyces (Glabrata group)	*Candida glabrata* CBS 138	CAGL0I00418g		
		Nakaseomyces bracarensis CBS 10,154	BN119_CABR0s04e02486g		
		Nakaseomyces delphensis CBS 2170	BN124_NADE-0s15e02486g		
		Nakaseomyces nivariensis CBS 9983	BN122_CAN-I0s20e01914g		
		Saccharomyces cerevisiae S288c	YGL055W		

Yeasts belonging to the same genus or group are reported within lines. Yeasts evolutionally aggregated in clades (CTG, KLE, ZT and WGD) are highlighted in bold. KLE and ZT clades were described in Marcet-Houbert and Gabaldón (2015). *K. lactis* genes used as query are marked in bold italics

Table 2), probably due to longer evolutionary distance. These values were actually lower than the identity value of Fad3 (62.4%). Although the existence of Δ15 desaturase without Δ12 desaturase is highly improbable, in these cases, confirmatory assays might be necessary to establish enzyme specificity because bifunctional Δ12/Δ15 desaturases are known in fungi (see for example Buček et al. 2014); however, only C18:2 FAs were detected in *Y. lipolytica* (Liu et al. 2015) indicating that the Omega desaturase in this yeast is Δ12.

Omega (Δ15) desaturases. The yeast species with Δ15 desaturase (Table 1) belonged only to the CTG clade (except *M. farinosa*) and to the entire *Lachancea* genus, in addition to the reference yeast *K. lactis*. Identity of these enzymes to Fad3 ranged from 65.6 to 82.7%. The high identity values between Δ12 and Δ15 desaturases suggests a common Δ12 ancestor from which Δ15 enzymes evolved (Wang et al. 2013). A phylogenetic analysis was performed using PhylomeDBv4, a program that provides phylomes reconstructed following a gene-based approach (Huerta-Cepas et al. 2011, 2014, http://phylomedb.org: a direct link to this program can be found in the GRYC site). The phylogenetic analysis of Omega desaturases confirmed this evolutionary hypothesis (Fig. 2).

Deletion of FA desaturases genes

Gene deletion or mutagenesis and phenotypic analysis of the resulting mutant strain is a diffused strategy to start the characterization and to assign a function to a protein or enzyme in yeast. The most commonly studied yeasts have been subjected to this approach. The successful generation of deleted strains indicates that the gene is not an essential one. This is the case of the deletion of a gene coding for uniquely biosynthetic enzymes that can be simply bypassed by the addition and assimilation of the biosynthetic product from the growth medium. However, if the enzymes have additional cellular functions, suppression by the biosynthetic product might not be effective or completely effective. If the additional function is important or essential for cellular fitness or viability, the deletion of the gene might be hard or impossible to be generated and selected.

S. cerevisiae has a single SCD gene *OLE1* (*YGL055W*) whose deletion caused oleate/palmitoleate auxotrophy (Stukey et al. 1989). A prolonged UFAs deprivation induced an increased frequency of cell death but quiescent-remaining cells recovered wild type growth upon UFAs supplementation. Other interesting phenotypes are associated to *OLE1* mutations, like mitochondrial dynamics defects (Stewart and Yaffe 1991). *Candida albicans* has

Table 2 Sizes and identities of the yeast Desaturases

Δ9 coding gene	Size	Identity	Δ12 coding gene	Size	Identity	Δ15 coding gene	Size	Identity
KLLA0C05566g	478	478/478(100.00%)	KLLA0F07095g	411	411/411(100.00%)	KLLA0B00473g	416	416/416(100.00%)
SAKL0A07326g	481	391/477(81.97%)	SAKL0H03872g	417	293/403(72.70%)	SAKL0A06556g	420	335/407(82.31%)
KLTH0H06798g	489	394/477(82.60%)	LAFE0E07140g	425	292/395(73.92%)	LACI0G19350g	413	331/410(80.73%)
LANO0F02872g	489	394/478(82.43%)	LADA0E11870g	414	287/407(70.52%)	LAQU0S16e00650g	425	333/413(80.63%)
LAQU0S02e09736g	481	394/477(82.60%)	LANO0H18074g	421	280/393(71.25%)	LADA0A03312g	415	334/404(82.67%)
LACI0B05468g	487	382/477(80.08%)	LACI0G04676g	421	283/392(72.19%)	KLTH0E10912g	447	332/413(80.39%)
LAMI0G09076g	487	384/477(80.50%)	LAQU0S01e03378g	419	278/401(69.33%)	LAFA0C03928g	412	328/402(81.59%)
LADA0A03994g	492	381/477(79.87%)	KLTH0D13640g	419	279/401(69.58%)	LAFE0C06612g	413	329/412(79.85%)
LAFE0C07360g	487	390/477(81.76%)	LAWA0E13432g	422	276/401(68.83%)	LANO0F02212g	419	332/413(80.39%)
LALA0S07e00716g	533	384/482(79.67%)	ZYBA0S07-01530g	418	272/408(66.67%)	LAMI0F08526g	416	320/403(79.40%)
LAFA0C04676g	499	382/487(78.44%)	AGOS_AFR589C	414	265/410(64.63%)	LAME0C07558g	418	326/419(77.80%)
ZYBA0S02-13410g	482	369/477(77.36%)	LAFA0D09868g	446	269/394(68.27%)	LAWA0H04896g	418	320/418(76.56%)
LAME0C06876g	501	383/487(78.64%)	LALA0S01e11056g	438	270/402(67.16%)	LALA0S13e03730g	417	325/408(79.66%)
AGOS_AAR153C	479	364/477(76.31%)	ZYRO0B07414g	416	270/416(64.90%)	CYFA0S01e00826g	423	284/415(68.43%)
ZYRO0C05016g	484	363/477(76.10%)	LAME0F06942g	425	262/386(67.88%)	CPAR2_801860	433	280/427(65.57%)
YGL055W	511	337/480(70.21%)	LAMI0A03136g	409	259/391(66.24%)	C1_13070C_A	434	273/389(70.18%)
CAGL0I00418g	491	328/476(68.91%)	DEHA2G05346g	417	264/389(67.87%)	DEHA2E14542g	436	276/411(67.15%)
BN123_ NABA0s37e00418g	499	322/477(67.51%)	CYFA0S32e00958g	418	255/394(64.72%)			
BN119_ CABR0s04e02486g	497	317/472(67.16%)	CPAR2_603730-	427	255/392(65.05%)			
CYFA0S04e03422g	482	317/477(66.46%)	C6_01110W_A	437	255/397(64.23%)			
CYFA0S10e01838g	477	322/479(67.22%)	KUCA_T00005823001	411	230/405(56.79%)			
BN124_NADE- 0s15e02486g	495	316/476(66.39%)	PISO0C09088g	426	225/384(58.59%)			
KNAG_0F03250-	490	315/475(66.32%)	PISO0D09155g	426	225/384(58.59%)			
BN122_CAN- I0s20e01914g	497	319/476(67.02%)	ARAD1D11396g	414	232/407(57.00%)			
BN121_ CACA0s33e03014g	497	313/474(66.03%)	YALI0B10153g	420	225/384(58.59%)			
NCAS_0D03250	498	309/478(64.64%)	YALIA101S01e13674g	420	225/384(58.59%)			
TBLA_0C06220	495	299/476(62.82%)						
KUCA_T00002566001	489	306/481(63.62%)						
C1_08360C_A	487	291/470(61.91%)						
DEHA2F03872g	485	287/471(60.93%)						
PISO0I17894g	486	289/474(60.97%)						
PISO0J19655g	486	285/474(60.13%)						
CPAR2_206900	487	287/481(59.67%)						
LAWA0H05556g	347	286/339(84.37%)						
YALI0C05951g	483	236/408(57.84%)						
YALIA101S01e24058g	483	236/408(57.84%)						
ARAD1D01914g	486	233/425(54.82%)						
CYFA0S07e03004g	474	198/413(47.94%)						
KUCA_T00001427001-	492	196/406(48.28%)						

The coding gene names of the yeast Desaturases are reported (first column) together with the deduced protein size (number of amino acids; second column). Identities are reported as the ratio of identical matches between each desaturase and the *K. lactis* desaturase (third column). Increased size values of the *K. lactis* reference derived from gap inclusion to maximize alignments. Ratios calculated as percentages are reported in parentheses

two SCD genes: C1_08360C_A and C2_07090C_A, corresponding to the *OLE1* and *OLE2* genes, respectively. In this organism, which is a dimorphic pathogen yeast, a homozygous deletion of *OLE1* couldn't be generated suggesting an essential role of this gene (Krishnamurthy et al. 2004). Altered *OLE1* expression caused impaired filamentous growth and chlamydospore formation, in addition to UFAs auxotrophy. Wild type phenotypes were recovered by UFAs supply and/or proper *OLE1* expression suggesting a close correlation between morphogenesis and membrane composition. No phenotype was associated to the deletion of *OLE2*, indicating a marginal role of the second

Fig. 2 Phylogenetic tree of Omega desaturases. The output of PhylomeDBv4 analysis is reported: analysis started with a homology search in the 34 fully-sequenced yeast genomes of the GRYC database using *K. lactis* KLLA0B00473g (*green dot*) *FAD3* gene as seed sequence. Duplications are marked in *red*. Speciation events are marked in *blue*. (Color figure online)

SCD in essential biological functions of this yeast. *Candida parapsilosis* also has two SCD genes, CPAR2_206900 and CPAR2_406570. Differently from *C. albicans*, the *OLE1* gene (CPAR2_206900) could be deleted to generate homozygous mutant strains which resulted sensitive to osmolytes, oxidative stress (H_2O_2), Sodium Dodecyl Sulphate (SDS) and with changed host-pathogen responses, in addition to UFAs auxotrophy (Nguyen et al. 2011). SCD genes have been deleted also in the yeast *Pichia pastoris* (*Komagataella pastoris*), not included in the GRYC database (Yu et al. 2012b; Zhang et al. 2015). In this yeast, the deletion of both SCD genes (named *FAD9A* and *FAD9B*) caused UFAs auxotrophy while only the deletion of *FAD9A* induced resistance to osmostress and SDS. The increased susceptibility or resistance to stressing substances and conditions in the SCD mutant strains might be explained by changed biochemical properties of membranes, as fluidity and/or permeability, caused by changes of FAs composition. Deletion of the single SCD gene (*KlOLE1*) has been attempted also in *K. lactis* without success (De Angelis et al. 2016) suggesting that, similarly to *C. albicans*, the SCD gene is essential also in this yeast.

The deletion of Omega desaturases genes has been reported for *C. albicans, P. pastoris, Lachancea kluyveri, K. lactis* and *Hansenula polymorpha*, the latter yeast not included in GRYC. No phenotypes were reported for *C. albicans* deletion mutants of *CaFAD2* and *CaFAD3* genes (Murayama et al. 2006). Slow growth phenotype at 15° and 30 °C and in the presence of ethanol was recorded for the deletion of *FAD12* (*FAD2*) gene in *P. pastoris* (Yu et al. 2012b). The deletion of *FAD12* resulted also in increased resistance to H_2O_2 and increased ROS content in this yeast (Zhang et al. 2015). Similarly to the deletion of SCD genes, phenotypes of *P. pastoris FAD12* deleted strains might be ascribed to changes of membrane properties. No phenotype was associated to the deletion of *FAD15*. In *K. lactis*, phenotyping revealed only a reduced growth at 8 °C caused by the deletion of *FAD2*, while no phenotypes were associated to *FAD3* deletion (De Angelis et al. 2016). Phenotypic analysis was not reported for the deletion of *FAD2* and *FAD3* in *L. kluyveri* and *H. polymorpha* (Watanabe et al. 2004; Oura and Kajiwara 2004; Sangwallek et al. 2014).

Deletion analysis indicate that the major growth defects (ranging from the simple UFAs auxotrophy, caused by the absence of basic Δ9 desaturase activity, to a more relevant cellular function essential for cell viability) result from the deletion of SCD genes, as also suggested by the unsuccessful attempt to generate SCD null mutants in *C. albicans* and *K. lactis*. Palmitoleic and oleic acids seem thus to be necessary and sufficient to ensure proper cellular fitness to yeasts in the routine conditions and media tested for basic research. When present, the second SCD genes seem to have a minor role. As far as Omega Desaturases are concerned, only Fad2 (Δ12) desaturase seem to contribute effectively to cell functions, as emerged from phenotypic analyses.

Transcription regulation of desaturases genes

Early studies on transcription regulation of desaturase genes were performed on *S. cerevisiae OLE1* gene. The effects of UFAs, of low temperature (10–15 °C) and of low oxygen tensions were preferentially studied. Promoter analysis allowed to identify sequences responsive to UFAs (Bossie and Martin 1989; McDonough et al. 1992; Choi et al. 1996) and low oxygen (Kwast et al. 1999; Nakagawa et al. 2001; Vasconcelles et al. 2001). Transcription of *OLE1* was repressed by UFAs (the products), and induced by FAs (the substrates) to some extent. Low temperature induced *OLE1* transcription thus favoring, consequently, fluidization of membranes by increasing UFAs content (Nakagawa et al. 2002). Hypoxia (low oxygen tension) also induced transcription of *OLE1*, but the connection between this environmental condition and membrane composition is not immediate. The increased expression of the desaturase in hypoxia might be necessary to compensate the reduced availability of the O_2 substrate or to ensure resistance to the accumulation of ethanol (Alexandre et al. 1994), which is the favored end-product of hypoxic metabolism (fermentation). SCD gene transcription has been studied also in other yeasts with comparable findings. In *K. lactis, KlOLE1* transcription was induced by hypoxia and ethanol (Micolonghi et al. 2012; De Angelis et al. 2016). In *P. pastoris, FAD9A* was induced by low temperature and repressed by UFAs (Yu et al. 2012a). *FAD9A* transcription was also induced by hydrogen peroxide (Zhang et al. 2015). Transcription of *OLE1* was repressed by UFAs in *Cryptococcus curvatus* (Meesters and Eggink 1996) while in *H. polymorpha* (Lu et al. 2000) and *Saccharomyces* (now *Lachancea*) *kluyveri* (Kajiwara 2002) a small repression or no effect on *OLE1* expression by UFAs were reported, respectively. Other studies were addressed to carbon source regulation; in *C. parapsilosis, OLE1* was induced by glucose (Pereira et al. 2015).

Expression of Omega Desaturases further modulates membrane fluidity by providing PUFAs. However, Omega Desaturases might respond differently to environmental conditions. For example, *FAD2* was induced and *FAD3* repressed by hypoxia in *K. lactis. FAD3* was also repressed by low temperature, but induced by ethanol (De Angelis et al. 2016). Low temperature induced Δ12 desaturase transcription in *Rhodotorula glutinis* (He et al. 2015). In *L. kluyveri*, low temperature induced both *FAD2* and *FAD3* transcription while UFAs had no effect (Watanabe et al. 2004; Oura and Kajiwara 2004). In *H. polymorpha*, UFAs repressed only *FAD3* transcription, but both *FAD2* and *FAD3* were induced by hypoxia (Sangwallek et al. 2014). Carbon source regulation of Omega Desaturase (Δ12) was studied in *Ashbya* (*Eremothecium*) *gossypii*, resulting in glucose induction of transcription and repression by soybean oil (Ledesma-Amaro et al. 2014).

In general, transcription regulation of desaturase genes and phenotypes caused by desaturase genes deletion can be functionally connected because gene regulation by a chemical or physical factor might correspond to sensitivity/resistance of the deleted strain to the same factor. However, although the environmental factors usually assayed are all effective in transcription modulation of desaturase genes, responses and/or adaptation to changes might act differently in different yeasts. Evolutionary history and specific niches might have contributed to this subtle diversification.

Regulation by Mga2

Mechanisms of transcription regulation of yeast desaturase genes have not been studied in detail except for the involvement of Mga2/Spt23-homologous proteins. These transcription factors are encoded by the ohnologue pair of genes *MGA2* and *SPT23* in *S. cerevisiae* in which their contribution to the regulation of *OLE1* has been studied extensively. They are ER-bound proteins activated by proteasomal proteolysis (Hoppe et al. 2000) and mediating the transcriptional response of *OLE1* to UFAs, low temperature and hypoxia (Chellappa et al. 2001; Jiang et al. 2002) by inducing/repressing transcription and/or affecting mRNA stability (Kandasamy et al. 2004; Jiang et al. 2001). Interestingly, the double deleted strain *mga2*Δ-*spt23*Δ has the same auxotrophic requirement for UFAs as the *ole1* mutant strains, while the single deleted strains are viable (Zhang et al. 1999; Chellappa et al. 2001).

Mga2/Spt23 proteins and genes have been studied also in other yeasts—*K. lactis, C. albicans, P. pastoris, Y. lipolytica*—included the fission yeast *Schizosaccharomyces pombe* (Micolonghi et al. 2012; Oh and Martin 2006; Yu et al. 2012a; Liu et al. 2015; Burr et al. 2016). Investigations have been especially addressed to lipid biosynthesis and regulation of desaturases. However, in *K. lactis*, also other aspects have been studied, in particular fermentative and respiratory metabolisms (Ottaviano et al. 2015). In all these yeasts a single gene copy was present, coding for Mga2/Spt23 proteins, and in all cases, except for *Y. lipolytica*, deleted strains showed reduced growth rates. This defect was compensated by the addition of UFAs in the medium, indicating a relevant role of these proteins in desaturases' gene regulation. In fact, transcription of the SCD and /or the Δ12 desaturase genes was affected in the deleted Mga2/Spt23 mutants of *K. lactis, C. albicans, P. pastoris* and *S. pombe*. Also the role of these regulatory factors in responses to environmental signals, such as low oxygen, low temperature and

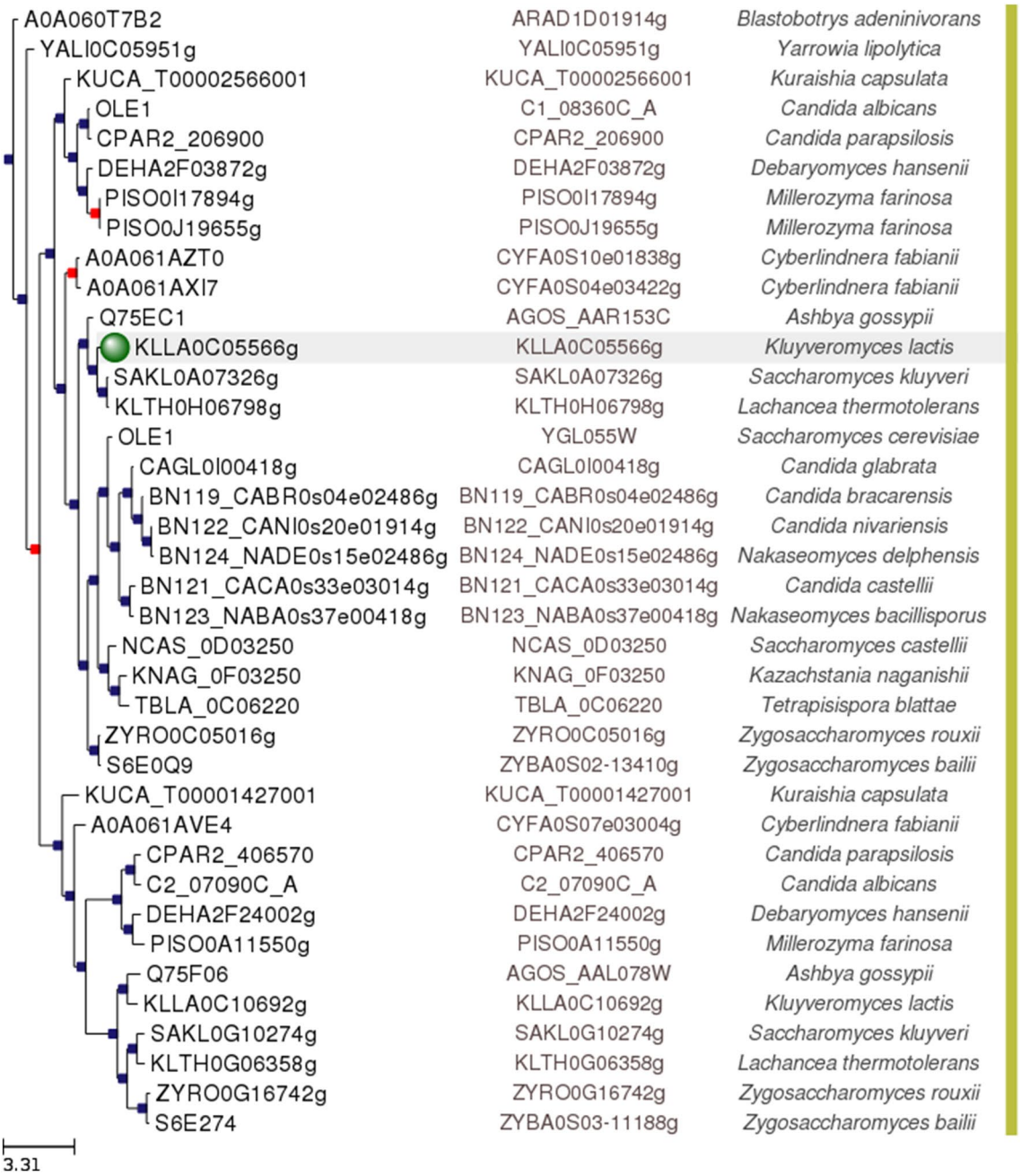

Fig. 3 Phylogenetic tree of SCD desaturases. The PhylomeDBv4 analysis was performed using *K. lactis* KLLA0C 05566 g (*green dot*) *KlOLE1* gene as seed sequence in the GRYC database. PSD group is represented by the branch including Q75F06, KLLA0C10692g, SAKL0G10274g, KLTH0G06358g, ZYRO0G16742g and S6E274. Duplications are marked in *red*. Speciation events are marked in *blue*. (Color figure online)

UFAs, has been studied. Interestingly, studies on Mga2 factor in *Y. lipolytica* showed opposite effects on desaturases gene expression and lipid composition compared to the other studied yeasts, suggesting a repressive activity of Mga2 on *OLE1* and *Δ12* genes (Liu et al. 2015).

Putative desaturases with weak similarity to SCD

Phylogenetic analysis of *KlOLE1* gene (KLLA0C05566g, Fig. 3) showed the occurrence of a gene duplication that generated SCD genes and a second group of genes. *OLE2* and second copies of *OLE1* genes were elements

of the second group and, as reported above, were already described, although a functional role and contribution of the SCD desaturases encoded by these genes to FAs biosynthesis has not been studied in detail to date. A distinct branch of these genes included *K. lactis* KLLA0C10692g. We used this gene to align proteins from GRYC database and we could select a sharply defined group of proteins sharing 50–58% identity over the whole protein length (KLLA0C10692g is 521 amino acids long). Ole2 and second Ole1 proteins were not in this group. These Putative SCD Desaturases (PSD) were all belonging to species of the KLE and ZT clades (*Lachancea* group, *Zygosaccharomyces, Eremothecium* and *Kluyveromyces* yeasts). SMART (http://smart.embl-heidelberg.de/) analysis of PSD proteins revealed that all of them contained a Cyt-b5 domain, suggestive of a redox catalytic activity, but an unambiguous desaturase domain was not always present and some PSD also lack trans-membrane domains (not shown). For example, *K. lactis* PSD contained only the Cyt-b5 domain and a GAF (domain present in phytochromes and cGMP-specific phosphodiesterases) domain. The occurrence of PSDs in a well defined taxonomic group of yeasts (all of them have Omega Desaturases but they are distinct from CTG clade) indicates a specific evolutionary role of these proteins, but the biochemical function and metabolic/physiological role of these enzymes remain to be elucidated by further analysis.

Conclusive remarks

Our overview on yeast Fatty Acids Desaturases showed that various evolutionary events generated duplication, deletion and functional differentiation of desaturase genes, suggesting that diversification of the composition of unsaturated FAs allowed adaptation to different environment of individual yeast species. However, only MUFAs seem to be essential for yeast viability, at least in standard laboratory conditions. The same environmental signals and shared mechanisms govern transcription of desaturase genes among yeasts. In pathogenic *Candida* yeasts, correlations between FA composition, FA desaturases and infective functions could be proved.

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