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Regulation of yeast fatty acid desaturase in response to iron deficiency

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ABSTRACT

Unsaturated fatty acids (UFA) are essential components of phospholipids that greatly contribute to the biophysical properties of cellular membranes. Biosynthesis of UFAs relies on a conserved family of iron-dependent fatty acid desaturases, whose representative in the model yeast *Saccharomyces cerevisiae* is Ole1. *OLE1* expression is tightly regulated to adapt UFA biosynthesis and lipid bilayer properties to changes in temperature, and in UFA or oxygen availability. Despite iron deficiency being the most extended nutritional disorder worldwide, very little is known about the mechanisms and the biological relevance of fatty acid desaturases regulation in response to iron starvation. In this report, we show that endoplasmic reticulum-anchored transcription factor Mga2 activates *OLE1* transcription in response to nutritional and genetic iron deficiencies. Cells lacking *MGA2* display low UFA levels and do not grow under iron-limited conditions, unless UFAs are supplemented or *OLE1* is overexpressed. The proteasome, E3 ubiquitin ligase Rsp5 and the $Cdc48^{Npl4/Ufd1}$ complex are required for *OLE1* activation during iron depletion. Interestingly, Mga2 also activates the transcription of its own mRNA in response to iron deficiency, hypoxia, low temperature and low UFAs. *MGA2* up-regulation contributes to increase *OLE1* expression in these situations. These results reveal the mechanism of *OLE1* regulation when iron is scarce and identify the *MGA2* auto-regulation as a potential activation strategy in multiple stresses.

1. Introduction

Cellular membranes are an essential requirement for life. In eukaryotic organisms, membranes represent selective and dynamic boundaries from the environment and between different intracellular compartments. Biological membranes are composed mostly of proteins and lipids, and glycerophospholipids have a major impact on their biophysical properties. The composition of membranes varies among organisms, cellular types and intracellular organelles to achieve defined properties and functions. Cells adjust the proportion of the saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) of their lipid bilayers in response to multiple environmental stresses to maintain proper fluidity, lipid packing and water permeability. Defects in UFA production profoundly reorganize organelle abundance and morphology, which can lead in extreme cases to cell death. Furthermore, UFA overproduction may be harmful, limit cell division and cause necrosis (reviewed in [1]). Therefore, UFA biosynthesis has to be tightly regulated. In humans, lipid metabolism deregulation has been linked to obesity-induced morbidity, type II diabetes, schizophrenia, Alzheimer's disease and Parkinson's disease [2,3].

The budding yeast *Saccharomyces cerevisiae* has been used as a reliable model organism to study multiple aspects of eukaryotic lipid biology [4]. The yeast Ole1 Δ9-fatty acid desaturase (the denoted SCD family in mammals) is a conserved iron- and oxygen-dependent enzyme anchored to the endoplasmic reticulum (ER) membrane that catalyzes the irreversible *de novo* biosynthesis of mono-UFAs [palmitoleic (16:1) and oleic (18:1)], which represent > 70% of total fatty acids (FAs), from the corresponding CoA-activated SFAs [palmitic (16:0) and stearic (18:0)]. Ole1 function is essential in yeast, unless the medium is supplemented with UFAs (reviewed in [3,5,6]). *OLE1* expression is highly regulated by numerous stimuli, including carbon source, extracellular FAs, temperature, oxygen levels and metal ions. Thus *OLE1* expression

Abbreviations: BPS, bathophenanthroline disulfonic acid disodium; ChIP, chromatin immunoprecipitation; ER, endoplasmic reticulum; FA, fatty acid; FAS, ferrous ammonium sulfate; Pol II, polymerase II; OD, optical density; RT-qPCR, Reverse Transcription-quantitative real time Polymerase Chain Reaction; SFA, saturated fatty acid; TM, transmembrane; TR, transcription rate; UFA, unsaturated fatty acid

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increases in response to low oxygen and low temperature, but is suppressed by UFA supplementation [7–11]. Two homologous and partially redundant transcription factors, Spt23 and Mga2, regulate *OLE1* expression. They are synthesized as inactive ~120 kDa homodimeric precursors anchored to the ER membrane through their carboxy-terminal transmembrane (TM) helix domain. Proteolytic cleavage releases a ~90 kDa amino-terminal fragment that translocates to the nucleus and activates *OLE1* transcription in a response denoted as the OLE pathway [8,12]. Although some differences exist, the activation of both transcription factors requires specific ubiquitylation by E3 ligase Rsp5, processing by the proteasome, and mobilization from the ER membrane with the help of substrate-recruiting factor Ubx2 and segregase/chaperone complex $Cdc48^{Npl4/Ufd1}$ [7,8,13–18]. Whereas the *mga2Δ* and *spt23Δ* single mutants are viable, the *mga2Δspt23Δ* double mutant is lethal unless UFAs are added to the growth medium [7,19]. Although each transcription factor is sufficient for *OLE1* expression, Mga2 is the dominant factor that activates *OLE1* in response to hypoxia, low temperature, cobalt and nickel [7,10,20,21]. Recent results have demonstrated that dimeric Mga2 functions as the membrane sensor for lipid saturation [5]. Specifically, Mga2 uses the rotational orientation of its transmembrane helix to sense lipid packing in the ER and to control *OLE1* transcription. Thus a high proportion of ER membrane SFAs promotes an Mga2 TM helix rotational orientation that activates the OLE pathway, while an increase in UFA abundance stabilizes inactive Mga2 [5].

Budding yeast uses two partially redundant transcription factors, Aft1 and Aft2, to activate the transcription of a group of genes, collectively referred to as the iron regulon, in response to iron deficiency. The iron regulon includes metalloreductases that reduce extracellular Fe^{3+} to its more soluble Fe^{2+} form, and the high-affinity iron transport system composed of multicopper ferroxidase Fet3 and iron-permease Ftr1 (reviewed in [22,23]). Yeast cells also express low-affinity iron transporters, such as Fet4 and Smf1. Despite oxo-diiron being an indispensable cofactor for the catalytic activity of Δ9-fatty acid desaturase Ole1, very little is known about the mechanisms that regulate these enzymes in response to alterations in iron bioavailability and how it affects UFA biosynthesis. We have previously reported that addition of the Fe^{2+}-specific chelator bathophenanthroline disulfonic acid disodium (BPS) to growth medium allows *OLE1* transcript levels to increase [24]. In this study, we present evidence to support that Mga2 activates *OLE1* transcription in response to iron limitation. This regulatory mechanism is essential for growth under iron-deficient conditions unless UFAs are added to the medium or *OLE1* is ectopically expressed. In mechanistic terms, ER-embedded Mga2 transcription factor activation by iron depletion seems a consequence of alterations in the relative FA composition of yeast membranes. Interestingly, we also reveal an Mga2 transcriptional auto-regulatory mechanism in response to iron deficiency, hypoxia and low temperatures or UFAs that contributes to enhance *OLE1* expression.

2. Material and methods

2.1. Yeast strains, culture conditions, and plasmids

The yeast strains used in this study are listed in Table 1. We used the pFA6a-13Myc-KanMX6 plasmid as a template [26], and specific oligonucleotides, to generate an integrative cassette for tagging the genomic copy of *OLE1* with the 13xMyc epitope at the carboxyl terminus (SPY901 yeast strain). Yeast precultures were incubated overnight at 30 °C in liquid synthetic complete SC medium [0.17% (w/v) yeast nitrogen base without amino acids and without ammonium sulfate (Pronadisa), 0.5% (w/v) ammonium sulfate (Panreac), 2% (w/v) glucose (Panreac), and 2 g L^{-1} Kaiser drop-out (Formedium)] lacking specific requirements whenever necessary, and reinoculated at an optical density at 600 nm (OD_{600}) of 0.2. To regulate iron availability in liquid cultures, cells were incubated for 6 h at 190 rpm in SC medium (+Fe) or SC supplemented with 100 μM BPS (Sigma) (−Fe). The Fe^{2+}-specific chelator ferrozine (Sigma) was used at the indicated concentrations to limit iron bioavailability in 2% agar (Pronadisa) solid media. Fatty acids (oleic and linoleic acids, Sigma) were added to a final 1 mM concentration and stabilized with 1% Tergitol Nonidet P-40 (Sigma). To induce P_{GAL1}-*OLE1* expression, glucose in SC was replaced with 2% galactose. For the spot assays, yeast cells were grown to the exponential phase, spotted in 10-fold serial dilutions starting at on OD_{600} of 0.1, and incubated at 30 °C for 3 days. The temperature sensitive strains were cultivated in SC at the permissive temperature of 25 °C and then transferred to the non-permissive temperature of 37 °C. After 1 h at 37 °C, 100 μM BPS was added, (−Fe) or not (+Fe), and cells were incubated for 5 h more. With the *npl4-1* mutant, 30 °C was used as the restrictive temperature [13]. For growth at low temperatures, yeast cells were collected, transferred to a pre-cold medium at 10 °C and incubated for 1 h. Hypoxia was achieved by bubbling nitrogen at a constant pressure for 5 h.

Table 1
List of yeast strains and plasmids used in this study.

Strain or plasmid	Description	Source
Strains		
HTLU-2832-1B	W303 *MATa HIS3, TRP1, LEU2, URA3, ADE2, can1*	F. Cross
W303-1A	*MATa, ura3-1, ade2-1, trp1-1, his3-11,15, leu2-3,112*	E. Herrero
MML1088	W303-1A *aft1Δ5 aft2::KanMX4*	E. Herrero
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Invitrogen
SPY386	BY4741 *fet3::URA3 fet4::KanMX4*	[25]
SPY824	BY4741 *mga2::KanMX4*	Invitrogen
SPY823	BY4741 *spt23::KanMX4*	Invitrogen
YWO0607	*MATa ura3 leu2-3,112 his3-11,15 Gal+*	D. H. Wolf
YWO0608	YWO0607 *pre1-1*	D. H. Wolf
YWO1	*MATa his3-Δ200, leu2-3,112, lys2-801, trp1-1(am), ura3-52*	[8]
Y0356	YWO1 *rsp5::HIS3; ura3-52::RSP5::URA3*	[8]
Y0358	YWO1 *rsp5::HIS3; ura3-52::rps5-2::URA3*	[8]
PSY580	*MATa ura3-52 leu2Δ1 trp1Δ63*	[13]
PSY2340	PSY580 *npl4-1*	[13]
PSY3074	*MATa his4-519, ura3-52, ade1-100, leu2-3,112 ufd1-1*	[13]
SPY901	BY4741 *OLE1-13xMyc::KanMX6*	This study
Plasmids		
pRS316-P_{GAL1}-OLE1	CEN *URA3* P_{GAL1}-*OLE1*	[8]
pPS2364	CEN *URA3 MGA2*	[13]
pPS2358	CEN *URA3 MGA2-truncated*	[13]
YEplac181-3HA-MGA2	2μ *LEU2* 3HA-*MGA2*	[8]
YCp33RNR4Z	CEN *URA3* P_{RNR4}-*lacZ*	R. S. Zitomer
pSP1039	CEN *URA3* P_{MGA2}-*lacZ*	This study
pFA6a-13Myc-KanMX6	Integrative *KanMX6*	([26]

All the plasmids used in this study are listed in Table 1. To construct the pSP1039 plasmid, 470 base pairs were amplified from the promoter region of the *MGA2* gene with oligonucleotides MGA2-470F-*Hin*dIII and MGA2-lacZ-*Pst*I-R (see Table 2), and then the PCR product was digested with restriction enzymes HindIII (Roche) and PstI (Roche). The YCp33RNR4Z plasmid, previously digested with HindIII and PstI to remove the *RNR4* promoter, was used to clone the *MGA2* promoter fused to the *lacZ* reporter gene. PCR amplifications were performed with Phusion polymerase (Finnzymes), and the cloned insert was sequenced. One Shot TOP10 chemically competent *Escherichia coli* cells (Invitrogen) were used to isolate and propagate plasmids.

2.2. RNA analyses

Total RNA extraction and cellular mRNA levels were determined by RT-qPCR as previously described [27]. The primers used for RT-qPCR