MGA2 or *SPT23* Is Required for Transcription of the Δ9 Fatty Acid Desaturase Gene, *OLE1*, and Nuclear Membrane Integrity in *Saccharomyces cerevisiae*

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ABSTRACT

MGA2 and *SPT23* are functionally and genetically redundant homologs in *Saccharomyces cerevisiae*. Both genes are implicated in the transcription of a subset of genes, including Ty retrotransposons and Ty-induced mutations. Neither gene is essential for growth, but *mga2 spt23* double mutants are inviable. We have isolated a gene-specific activator, *SWI5*, and the Δ9 fatty acid desaturase of yeast, *OLE1*, as multicopy suppressors of an *mga2*Δ *spt23* temperature-sensitive mutation (*spt23-ts*). The level of unsaturated fatty acids decreases 35–40% when the *mga2*Δ *spt23-ts* mutant is incubated at 37°. Electron microscopy of these cells reveals a separation of inner and outer nuclear membranes that is sometimes accompanied by vesicle-like projections in the intermembrane space. The products of Ole1p catalysis, oleic acid and palmitoleic acid, suppress *mga2*Δ *spt23-ts* and *mga2*Δ *spt23*Δ lethality and restore normal nuclear membrane morphology. Furthermore, the level of the *OLE1* transcript decreases more than 15-fold in the absence of wild-type Mga2p and Spt23p. Our results suggest that Mga2p/Spt23p control cell viability by stimulating *OLE1* transcription.

IN the budding yeast *Saccharomyces cerevisiae*, insertion of a Ty retrotransposon into the promoter region of a gene often results in alteration of the transcription of that gene (reviewed by Winston 1992). Several key genes required for general transcription have been identified as extragenic suppressors of Ty-induced promoter mutations and are named *SPT* (*S*uppressor of *T*y) genes. These include genes encoding the TATA-binding protein TBP (*SPT15*; Eisenmann *et al.* 1989), histone core proteins H2A and H2B (*SPT11* and *SPT12*; Clark-Adams *et al.* 1988), and proteins involved in transcription elongation by RNA polymerase II (*SPT4*, *SPT5*, and *SPT6*; Hartzog *et al.* 1998).

SPT23 was isolated as a multicopy suppressor of Ty-induced promoter mutations (Burkett and Garfinkel 1994). *SPT23* shares considerable homology with *MGA2*, a gene identified as a multicopy suppressor of a *snf2*-imposed block on *STA1* expression in *S. cerevisiae* var. *diastaticus* (Zhang *et al.* 1997). Although null mutations in either of these genes have only modest effects on cell growth, *mga2 spt23* double mutants are inviable. Because previous work suggests that Mga2p and Spt23p are transcriptional coactivators with overlapping specificities, we have been interested in identifying genes that are functionally related to or controlled by Mga2p/Spt23p. Therefore, we screened a multicopy plasmid library to identify suppressors of the cellular lethality resulting from the absence of Mga2p/Spt23p. The strongest plasmid-borne suppressor of *mga2*Δ *spt23-ts* lethality isolated from the screen is *OLE1*, which encodes the Δ9 fatty acid desaturase of yeast.

The fatty acid synthesis pathway in *S. cerevisiae* has been established using biochemical and genetic approaches (Figure 1; Paltauf *et al.* 1992; Schneiter *et al.* 1996; Schneiter and Kohlwein 1997). Yeast cells utilize exogenous free fatty acids through activation by at least four acyl-coenzyme A (CoA) synthetases (Faa1p–Faa4p; Johnson *et al.* 1994) or synthesize saturated fatty acids *de novo* to form the major saturated long chain fatty acids (SFAs) palmitoyl-CoA (16:0) and stearoyl-CoA (18:0). Three enzyme systems participate in this process: acetyl-CoA carboxylase (Acc1p), fatty acid synthetase (Fas1p and Fas2p), and elongase (Elo1p). A small portion of the long chain fatty acids can also be elongated to form very long chain fatty acids (C20-C30; Welch and Burlingame 1973).

In animal and fungal cells, monounsaturated fatty acids (UFAs) are synthesized from fatty acid precursors by an aerobic microsomal enzyme system that includes cytochrome b5, NADH-dependent cytochrome b5 reductase, and Δ9 fatty acid desaturase (Dailey and Strittmatter 1978, 1980; Stukey *et al.* 1990). The *OLE1* gene encodes the sole Δ9 fatty acid desaturase in *S. cerevisiae* and is essential for viability (Stukey *et al.* 1989, 1990). Ole1p catalyzes the formation of a double

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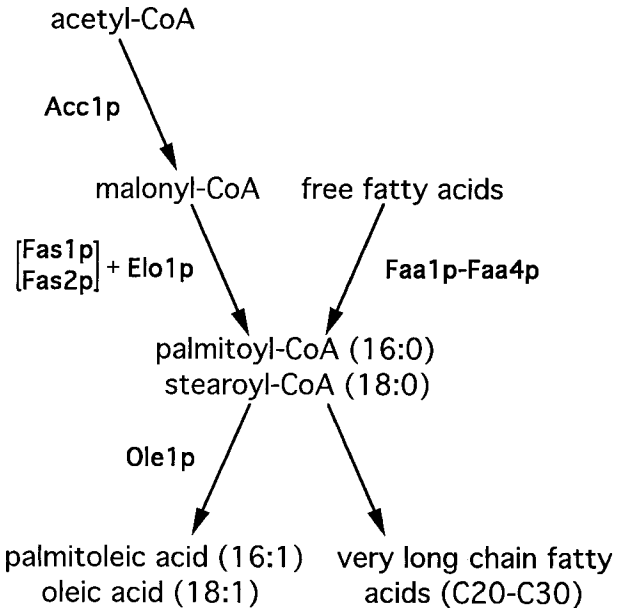

Figure 1.—Fatty acid biosynthesis in yeast. Relevant enzymes are shown.

bond between C9 and C10 of palmitoyl-CoA (16:0) and stearoyl-CoA (18:0), forming palmitoleic acid (16:1) and oleic (18:1) acid. Under laboratory conditions, yeast cells synthesize only monounsaturated fatty acids, which comprise about 70% of the total fatty acids in the cell. The correct ratio of saturated to unsaturated fatty acids is important for maintaining the optimal levels of membrane fluidity and curvature, which are essential for a variety of cellular processes (Krulwich *et al.* 1990; Carratu *et al.* 1996). Interestingly, an *OLE1* temperature-sensitive allele, *mdm2*, causes aberrant mitochondrial segregation at the nonpermissive temperature and UFAs decrease about 2.5-fold (McConnell *et al.* 1990; Stewart and Yaffe 1991).

The steady state level of *OLE1* mRNA is regulated by transcription and RNA turnover, and both regulatory processes are affected by the presence of fatty acids (Bossie and Martin 1989; Choi *et al.* 1996; Gonzalez and Martin 1996). Addition of an exogenous UFA represses the transcription of *OLE1* and promotes the decay of *OLE1* mRNA. *cis*-acting sequences in the *OLE1* 5′ noncoding region as well as *trans*-acting factors are implicated in the transcriptional regulation of *OLE1* (Choi *et al.* 1996). Two fatty acid activation genes, *FAA1* and *FAA4*, the transcriptional activator *HAP1*, and acyl-CoA-binding protein have been reported to regulate *OLE1* transcription. Our current studies suggest that Mga2p and Spt23p stimulate transcription of *OLE1*. Loss of functional Mga2p/Spt23p results in a lower level of UFAs and subsequent cell death. Plasmid-borne expression of *OLE1*, or UFAs present in the growth medium, rescues the conditional *mga2*Δ *spt23-ts* mutant at the nonpermissive temperature or a *mga2*Δ *spt23*Δ double mutant. Electron microscopy shows that the *mga2*Δ *spt23-ts* mutant has a morphologically altered nuclear membrane at the nonpermissive temperature, a defect that is also reversed by addition of UFAs. Therefore, our results suggest that *OLE1* is an essential target gene affected by Mga2p/Spt23p.

MATERIALS AND METHODS

Strains and media: Yeast strains are listed in Table 1. The *ole1::LEU2* mutant SZ66 was constructed by one-step gene disruption (Rothstein 1991) using a *Sac*I-*Hin*dIII fragment from pSRZ163. Correct gene replacement was verified by Southern blot analysis (S. Zhang, unpublished results). *OLE1-URA3* was integrated at the *ura3-52* locus of strains DG1555 and DG1667 by linearizing the YIp plasmid pSRZ176 at a unique *Stu*I site in *URA3*. Strain SZ67 was obtained by tetrad dissection of a diploid strain heterozygous for *spt23*Δ-*hisG* and *mga2*Δ*::LEU2* on an unsupplemented YPD plate, followed by replica plating to YPD containing unsaturated fatty acids (UFAs). Standard growth media were used as described by Rose *et al.* (1990). Media supplemented with UFAs were made by adding equimolar amounts of palmitoleic acid (16:1) and oleic (18:1) acid to a final concentration of 0.5 mm. Tergitol (Fluka Chemical, Buchs, Switzerland) was included at a final concentration of 1% (v/v) to solubilize the UFAs, as described by Stukey *et al.* (1989). Media supplemented with a saturated fatty acid (SFA) were made by adding palmitic acid to a final concentration of 1.2 mm. Brij 58 (Aldrich Chemical, Milwaukee) was added to a final concentration of 1% (w/v) to solubilize the SFA, as described by Mishina *et al.* (1980).

Plasmid and DNA manipulations: Plasmids were constructed by standard procedures (Sambrook *et al.* 1989). Generally, restriction fragments were purified from agarose gels by using glass milk (BIO 101, Inc., Vista, CA). Restriction enzymes [New England Biolabs (NEB), Beverly, MA], T4 DNA ligase (NEB), high-fidelity thermostable DNA polymerase (Boehringer Mannheim, Indianapolis), and the Klenow fragment of DNA polymerase I (NEB) were used according to the suppliers' suggestions. DNA sequencing was performed using a PRISM sequencing kit (Perkin-Elmer, Norwalk, CT). Plasmids were purified from *Escherichia coli* by a boiling lysis method (Holmes and Quigly 1981) and introduced into yeast cells as described by Gietz *et al.* (1992). Plasmid pBDG794, which contains the *spt23-ts* allele, was isolated after mutagenizing plasmid pSRZ47 with hydroxylamine (Zhang *et al.* 1997). Plasmids pSMS1, pSMS6, pSMS7, pSMS8, pSMS9, and pSMS22 were isolated as suppressors of the lethality conferred by *mga2*Δ *spt23-ts* at 37°. Plasmids pSRZ157 (2μ-*OLE1*), pSRZ161 (*CEN-OLE1*), and pSRZ176 were constructed by subcloning a 3.1-kb *Sac*I-*Hin*dIII fragment containing *OLE1* from pSMS7 into pRS426, pRS416, and pRS406 (Sikorski and Heiter 1989), respectively. Plasmid pSRZ159 (2μ-*ole1*) was constructed by digesting pSRZ157 with *Tth*111I, filling in the DNA ends with Klenow DNA polymerase, and self-ligation. The *ole1::LEU2* gene disruption in pSRZ163 was constructed by a homologous recombination event that replaced *OLE1* sequences from codons 14–498 in pSRZ161 with a 2.2-kb polymerase chain reaction (PCR) product containing the *LEU2* gene as described by Manivasakam *et al.* (1995). The relative copy number of the *URA3*-based low-copy plasmids pRS416 and *CEN-OLE1* in strains DG1555 and DG1667 was determined by Southern blot analysis following digestion with *Hin*dIII. Total yeast DNA from certain transformants was isolated after cells were shifted to 37° for 6 hr. The resulting filter was hybridized with a ^{32}P-labeled internal *Nde*I-*Stu*I fragment from *URA3*. The transformants contained two types of *Hin*dIII frag-

TABLE 1

Yeast strains

Strain	Genotype
DG1555	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*-hisG gal2*
DG1667	*MAT*α *his4-912*δ *lys2-61 ura3-52 leu2*Δ*-hisG trp1-hisG spt23*Δ*-hisG mga2*Δ*::LEU2 (CEN-TRP1-spt23-ts)*
SZ30	*MAT*α *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*-hisG spt23*Δ*-hisG mga2*Δ*::LEU2 (CEN-URA3-SPT23)*
SZ49	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*-hisG*
SZ60	*MAT*α *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*-hisG spt23*Δ*-hisG mga2*Δ*::LEU2 (CEN-URA3-MGA2)*
SZ66	*MAT***a** *his3-*Δ*200 lys2-128*δ *ura3 leu2*Δ*-hisG ole1*Δ*::LEU2*
SZ67	*MAT*α *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*-hisG spt23*Δ*-hisG mga2*Δ*::LEU2*

Strain DG1667 has been described previously (Zhang *et al.* 1997). All other strains are from this work.

ments that hybridize with this probe: cells harboring the pRS416 vector or the *CEN-OLE1* plasmid contained a 4.4- or 7.5-kb fragment, respectively, and a chromosomal 2.1-kb fragment from *ura3-52*. The ratio of the hybridization signals of the plasmid fragments to the signal of the 2.1-kb chromosomal *Hin*dIII fragment indicated the relative copy number of the plasmids. The copy number of the integrated pSRZ176 plasmid at the *ura3-52* locus in strains DG1555 and DG1667 was determined by Southern blot analysis after *Eco*RV digestion. The resulting filter was hybridized with a ^{32}P-labeled *URA3* probe as described above. The integrants contained two *Eco*RV fragments that hybridized with this probe: a 4.8-kb fragment spanning an external *ura3-52*/*URA3* chromosomal/plasmid recombination junction and a 6.2-kb fragment spanning an internal *URA3*/*URA3* plasmid/plasmid recombination junction. The ratio of the hybridization signal of the 6.2-kb fragment to that of the 4.8-kb fragment indicated the pSRZ176 copy number at the chromosomal *ura3-52* locus. Hybridization signals were quantitated by phosphorimaging using conditions suggested by the manufacturer (Molecular Dynamics, Inc., Sunnyvale, CA) and ImageQuant software (Version 1.1).

Lipid analysis: DG1555 and the DG1667-based strains were grown in either YPD or synthetic complete media lacking uracil (SC-Ura) to early log phase at 23°. The cultures were either allowed to continue growing at 23° or shifted to 37°, and samples were removed 6 and 15 hr after the temperature shift. Total lipids were extracted by a modification of standard methods (Bligh and Dyer 1959; Martin *et al.* 1981; Kates 1986). Briefly, yeast cells were broken by vortexing in the presence of glass beads in methanol:choloroform:water (2:1:0.8). Total lipids were saponified with sodium hydroxide, then esterified by transmethylation with boron trifluoride (Morrison and Smith 1964). The fatty acid methyl esters were analyzed by gas-liquid chromatography. Commercially available methyl esters of palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1), and oleic acid (18:1) (Sigma, St. Louis) were used as standards to identify and quantitate cellular fatty acids.

Electron microscopy: Strains DG1555 and DG1667 were grown to early log phase in YPD broth at 23°, then either kept at 23° in YPD or shifted to 37° in either YPD or YPD supplemented with UFAs, and grown for an additional 6 hr. The *ole1* mutant strain SZ66 was pregrown to early log phase in YPD medium supplemented with UFAs at 30°. The cells were washed with 1 and 0.5% tergitol to remove residual UFAs. The washed cells were incubated in either supplemented or unsupplemented YPD medium at 30° or 37° for 6 or 14.5 hr. Cells collected by vacuum filtration onto a 0.45-μm nitrocellulose filter (Millipore, Bedford, MA) were washed once with water and once with buffer A [40 mm KPO_4 (pH 6.5), 0.5 mm $MgCl_2$], then fixed overnight at 4° with 4% formaldehyde and 2% glutaraldehyde in buffer A. After two additional washes with buffer A, the cells were resuspended in a buffer containing 200 mm Tris-HCl (pH 9.0), 20 mm EDTA, and 100 mm β-mercaptoethanol, then incubated for 10 min at room temperature. The fixed cells were washed three times with 0.1 m KPO_4 (pH 7.5), digested with Zymolyase 100T (250 ng/ml final concentration; ICN Corp., Aurora, OH), and post-fixed with 1% osmium tetroxide by standard techniques (Byers and Goetsch 1991).

RNA analyses: Total yeast RNA was isolated and Northern filter hybridizations were performed as described by Schmitt *et al.* (1990) and Zhang *et al.* (1997), respectively. ^{32}P-labeled *OLE1*, *ACC1*, and *GAL80* hybridization probes were synthesized using the Megaprime DNA Labeling System (Amersham, Buckinghamshire, UK). The template for labeling *OLE1* was a 1.3-kb *Sal*I-*Pac*I fragment of pSRZ157. The template for the *GAL80* probe was a 1.3-kb genomic fragment amplified by PCR using primers 5′-CCACTCCCGTCATGGAC-3′ and 5′-GGGGGCCAAGCACAGG-3′. The template for the *ACC1* probe was a 1.9-kb genomic fragment amplified by PCR using primers 5′- CCGGACAAGGGCTTC-3′ and 5′-GTACCCCTTC CCACAG-3′. Hybridization signals were quantitated by phosphorimage analysis as described above. RNA differential display analysis (Liang and Pardee 1992) was performed using RNAimage (GenHunter Corp., Nashville, TN) under conditions suggested by the supplier. Residual DNA was removed from yeast RNA using MessageClean (GenHunter Corp.).

RESULTS

Isolation of plasmid-borne suppressors of *mga2*Δ *spt23-ts*: The synthetic lethality of an *mga2 spt23* double mutant suggests that these genes share functions that are essential for cell viability. To identify genes functionally related to or controlled by Mga2p/Spt23p, we screened multicopy plasmid libraries for clones that suppress the lethality of an *mga2*Δ *spt23-ts* mutant, DG1667, at the nonpermissive temperature of 37°. Strain DG1667 carries the *spt23-ts* allele on plasmid pBDG794 and contains chromosomal deletions of *MGA2* and *SPT23*; therefore, we used plasmid segregation to determine whether a given suppressor plasmid is dependent on *spt23-ts* for its activity. As expected, DG1667 grew well at 23° but not at 37° (Figure 2, vector), compared with the *MGA2*/*SPT23* wild-type strain DG1555 (Wt./vector). Two *URA3*-based multicopy yeast genomic libraries (Carlson and

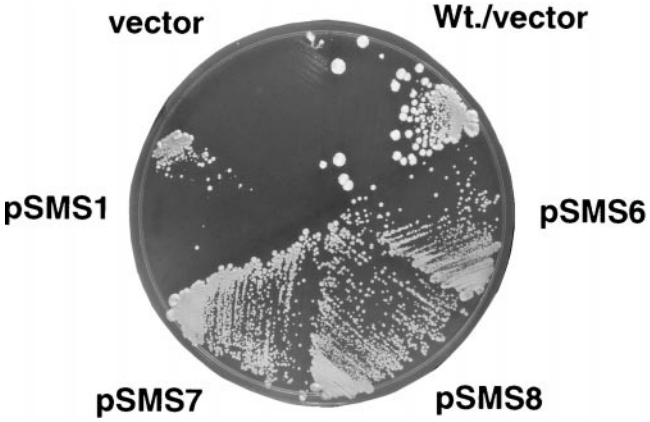

Figure 2.—Plasmid-borne suppressors of *mga2*Δ *spt23-ts*. Strain DG1667 (*mga2*Δ *spt23-ts*) containing pRS426 (vector), pSMS6 (*SPT23*), pSMS8 (*MGA2*), pSMS7 (*OLE1*), or pSMS1 (*SWI5*) was streaked on an SC-Ura plate and incubated for 4 days at 37°. The *MGA2 SPT23* wild-type strain DG1555 containing pRS426 (Wt./vector) is also shown.

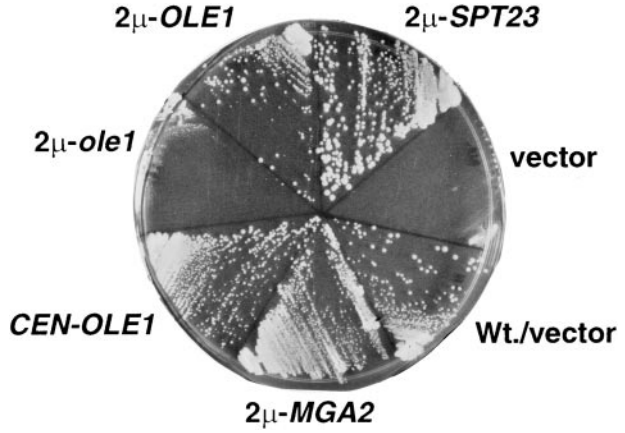

Figure 3.—Suppression of *mga2*Δ *spt23-ts* by plasmid-borne *OLE1*. Strain DG1667 (*mga2*Δ *spt23-ts*) containing 2μ-*SPT23* (a multicopy plasmid containing *SPT23*, pBDG769), vector (multicopy plasmid pRS426), 2μ-*MGA2* (pSRZ65), *CEN-OLE1* (a low-copy plasmid containing *OLE1*, pSRZ161), 2μ-*ole1* (a multicopy plasmid containing a mutated *ole1* gene, pSRZ159), or 2μ-*OLE1* (pSRZ157) was streaked on an SC-Ura plate and incubated for 4 days at 37°.

Botstein 1982; Nehlin *et al.* 1989) were introduced into DG1667 at 23° to allow all possible transformants to grow. Primary transformant colonies were then replica plated to fresh SC-Ura plates and incubated at 37°. A total of 20 transformants grew at 37° from more than 400,000 screened. Suppressors pSMS1, pSMS6, pSMS7, pSMS8, pSMS9, and pSMS22 were plasmid borne and restored growth at the nonpermissive temperature to various extents (Figure 2; S. Zhang, unpublished results). The suppression by the other 14 isolates probably resulted from chromosomal mutations; we will characterize these suppressors elsewhere.

To identify the suppressor genes, a partial DNA sequence from the genomic inserts of the six plasmid-borne suppressors was submitted to the *Saccharomyces* Genome Data Base (Stanford University). As expected, we reisolated *MGA2* twice (pSMS8 and pSMS9; in Figure 2, pSMS8 is shown) and *SPT23* once (pSM6). Strain DG1667 containing pSM6, pSM8, or pSMS9 grew well at 37°, compared with the DG1667 (vector) mutant and DG1555 (Wt./vector) wild-type control strains. The suppressor plasmids pSMS7 and pSMS22 contained overlapping inserts, as demonstrated by DNA sequence and restriction enzyme analyses (S. Zhang, unpublished results). The suppression by pSMS7 and pSMS22 was independent of *spt23-ts* and growth temperature, because the loss of plasmid pBDG794 did not affect the suppressor's activity at 23°, 30°, or 37°. The *spt23-ts*-independent suppression by pSMS7 and pSMS22 also showed that these plasmids suppressed lethality in an *mga2*Δ *spt23*Δ mutant. Deletion and site-directed mutagenesis of the insert in pSMS7 indicated that *OLE1* was responsible for the suppression (Figure 3; S. Zhang, unpublished results), because a subclone containing only the *OLE1* open reading frame (ORF) suppressed *mga2*Δ *spt23-ts* (2μ-*OLE1*) and a frameshift mutation in the *OLE1* coding sequence no longer suppressed *mga2*Δ *spt23-ts* (2μ-*ole1*). As expected, either 2μ-*MGA2* or 2μ-*SPT23* suppressed the temperature sensitivity of DG1667. Surprisingly, a low-copy centromere-based plasmid containing *OLE1* was also capable of suppression (*CEN-OLE1*).

Plasmid pSMS1 suppressed *mga2*Δ *spt23-ts* weakly (Figure 2) and the suppression was dependent on the *spt23-ts* plasmid, suggesting a possible interaction between the suppressor and Spt23p. Analysis of its genomic insert by DNA sequencing and mutagenesis indicated that *SWI5* conferred the suppressor activity (S. Zhang, unpublished results). *SWI5* encodes a transcriptional activator necessary for expression of the *HO* endonuclease gene and of *EGT2*, a gene required for mother-daughter cell disjunction (Breeden and Nasmyth 1987; Kovacech *et al.* 1996). Further analysis of the possible interaction between Spt23p and Swi5p will be presented elsewhere.

Fatty acid content and requirements in the *mga2 spt23* mutants: The Δ9 fatty acid desaturase encoded by *OLE1* is the only desaturase identified in *S. cerevisiae* (Stukey *et al.* 1989, 1990). Under standard laboratory growth conditions in minimal or rich media, yeast produce monounsaturated fatty acids (UFAs), which constitute about 70% of the total fatty acids in the cell. Because an *ole1* null mutant requires exogenous UFAs for growth and plasmid-borne *OLE1* expression suppresses the *mga2*Δ *spt23-ts* mutant, we reasoned that *OLE1* restored UFA synthesis at 37°. Therefore, we determined the saturated fatty acid (SFA; 16:0 and 18:0) and UFA (16:1 and 18:1) content in mutant and wild-type cells (Table 2), and determined whether the *mga2 spt23* mutants grew when supplemented with an SFA or UFAs (Figure 4). The wild-type (*MGA2 SPT23*) and mutant (*mga2*Δ

TABLE 2

Fatty acid levels

Relevant genotype	Temperature	Time (hr)	SFA and UFA content (%)				
			16:0	16:1	18:0	18:1	Total UFAs
MGA2 SPT23	23°		17.8	45.3	9.2	27.7	73
MGA2 SPT23	37°	6	21.5	33.1	10.5	34.9	68
MGA2 SPT23	37°	15	15.6	39.6	6.6	38.2	77.8
*mga2*Δ *spt23-ts*	23°		18.7	46.9	7.7	26.7	73.6
*mga2*Δ *spt23-ts*	37°	6	30.3	27.3	16.2	26.2	53.5
*mga2*Δ *spt23-ts*	37°	15	32.6	23.4	19.3	24.7	48.1
*mga2*Δ *spt23-ts* 2μ-*OLE1*	20°		9.6	39.2	8.4	42.8	82
*mga2*Δ *spt23-ts* 2μ-*OLE1*	37°	15	11.3	31.4	9.8	47.5	78.9

Total UFAs were obtained by adding the percentages of 16:1 and 18:1 UFAs.

spt23-ts) strains contained about 73% UFAs at 23°, and the wild-type cells contained 77.8% UFAs 15 hr after the shift to 37° (Table 2, total UFAs). However, the percentage of UFAs in the *mga2*Δ *spt23-ts* mutant decreased to 53.5% after a 6-hr incubation and to 48.1% after 15 hr at 37°. When UFA levels in the *mga2*Δ *spt23-ts* mutant at 23° and 37° were compared, a loss of almost half of the 16:1 UFA palmitoleic acid occurred after 15 hr at 37°. As expected for cells lacking Ole1p activity (*mga2*Δ *spt23-ts* at 37°), the levels of 16:0 and 18:0 SFAs increased to over 30 and 16%, respectively, at 37°. The presence of plasmid-borne *OLE1* in the mutant cells restored UFA synthesis to wild-type levels (2μ-*OLE1*).

We then determined whether direct supplementation of UFAs (16:1 and 18:1) to the growth medium suppressed the conditional lethality caused by *mga2*Δ *spt23-ts* and the unconditional lethality of the *mga2*Δ *spt23*Δ double deletion (Figure 4). Inclusion of the SFA palmitic acid or UFAs in the growth medium was mildly toxic to wild-type cells (Figure 4A), because these cultures reached stationary phase at a lower cell density. However, the supplemented cells maintained their normal shape, budding pattern, doubling time (80 min), and had no other obvious defects (S. Zhang, unpublished results). In contrast, the *mga2*Δ *spt23-ts* mutant only grew at the nonpermissive temperature of 37° in medium supplemented with UFAs (Figure 4B, 131 min doubling time). Addition of an SFA did not support growth, indicating that the suppression by UFAs was specific. Furthermore, supplementation with UFAs also suppressed the lethality of the *mga2*Δ *spt23*Δ null mutant at the standard growth temperature of 30° (Figure 4C, 120 min doubling time). Taken together, these results suggest that the absence of Mga2p and Spt23p results

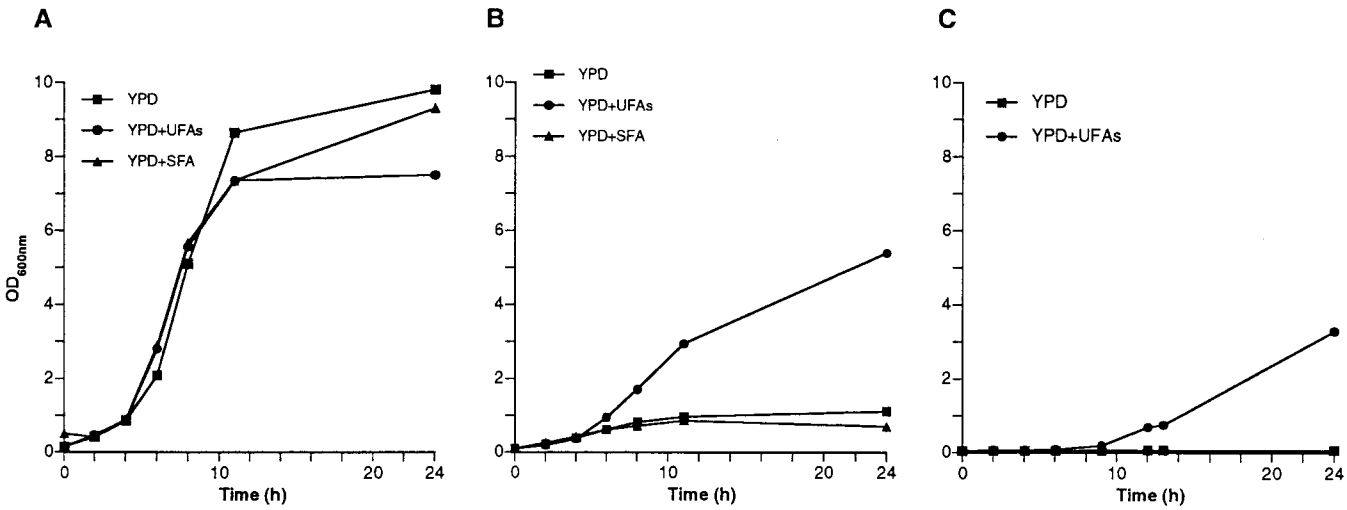

Figure 4.—UFA supplementation suppresses *mga2 spt23* mutants. Strains (A) DG1555 (*MGA2 SPT23*), (B) DG1667 (*mga2*Δ *spt23-ts*), and (C) SZ67 (*mga2*Δ *spt23*) were pregrown in either YPD at 23° (A and B) or YPD + UFAs at 30° (C) to early log phase. The cells were washed, resuspended in either YPD, YPD + 0.5 mm UFAs (equimolar amounts of palmitoleic and oleic acids), or YPD + 1.2 mm SFA (palmitic acid) and shifted to 37° (A and B) or kept at 30° (C). Cell growth was monitored by optical density (OD_{600nm}). Each measurement was the average from three experiments, and the doubling time for a given strain in each experiment was comparable (±15%).

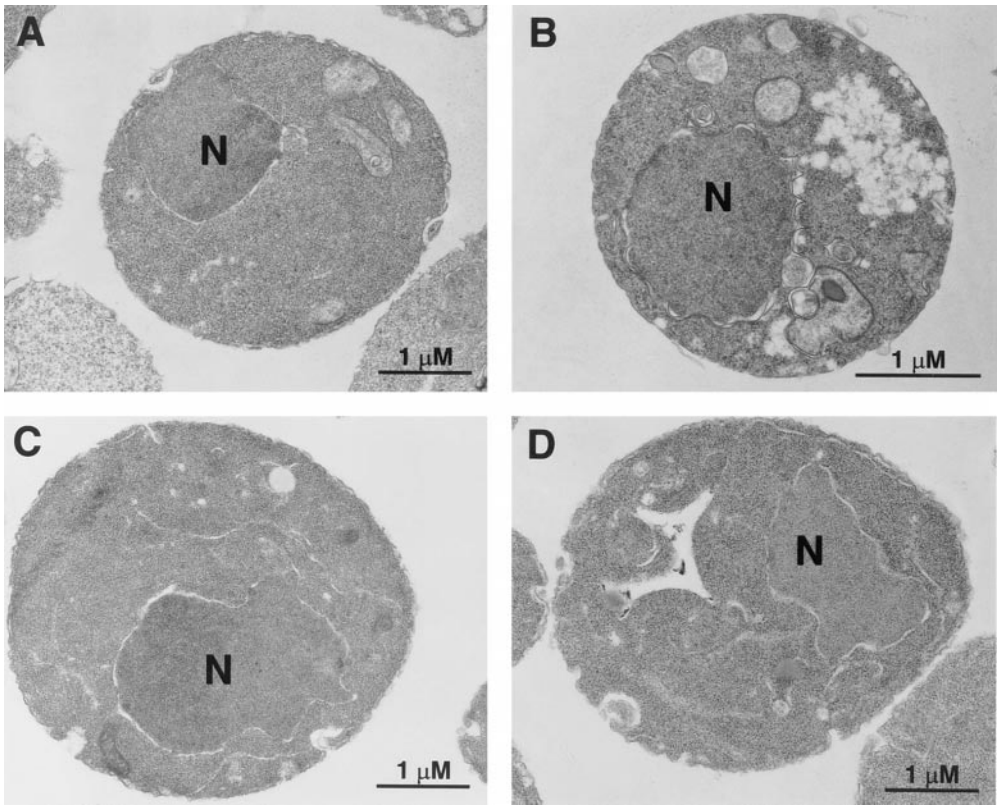

Figure 5.—Mga2p/Spt23p affect nuclear membrane morphology. (A) Wild-type DG1555 or (B–D) *mga2*Δ *spt23-ts* mutant DG1667 strains were pregrown to early log phase at 23° in YPD, then either shifted to (A and B) 37° in YPD or (C) YPD supplemented with 0.5 mm UFAs for 6 hr, or (D) kept at 23° for 6 hr. The cells were then fixed and prepared for electron microscopy. The nucleus (N) is indicated and magnification is ×20,000.

in a significant decrease in intracellular UFAs and subsequent cell death at 30° and 37°.

***MGA2/SPT23* and *OLE1* affect nuclear membrane morphology:** Certain fatty acid-deficient mutants have nuclear membrane defects. In particular, a mutation in the yeast acetyl-CoA carboxylase gene *acc1-7-1* causes severe alterations in nuclear envelope morphology (Schneiter *et al.* 1996). Therefore, we examined the cellular structure of the *mga2*Δ *spt23-ts* mutant (Figure 5) and an *ole1* null mutant (Figure 6) by electron microscopy. The *mga2*Δ *spt23-ts* mutant showed normal nuclear envelope morphology at 23° (Figure 5D), which was similar to that observed for the wild type at 23° (S. Zhang, unpublished results) or 37° (Figure 5A). The nuclear membrane in the *mga2*Δ *spt23-ts* mutant was severely distorted at 37° (Figure 5B). Like *acc1-7-1*, the *mga2*Δ *spt23-ts* mutant showed a separation of the inner and outer nuclear membranes and some cells contained vesicle-like structures in the intermembrane space. Addition of UFAs to the growth medium corrected the nuclear membrane defect in the *mga2*Δ *spt23-ts* mutant (Figure 5C).

Because the cell growth and morphological defects present in the *spt23 mga2* double mutants are reversed

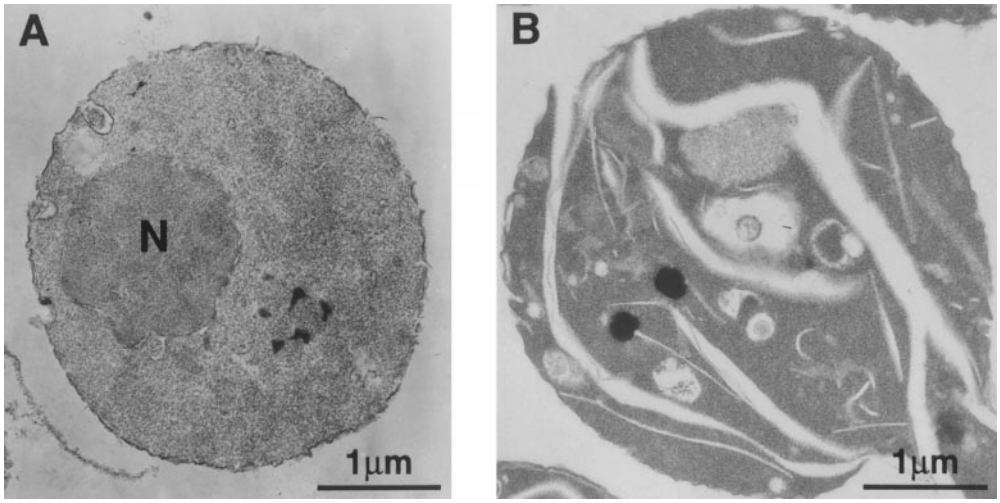

Figure 6.—Severe morphological defects of an *ole1* null mutant. Strain SZ67 was pregrown in YPD + UFAs to early log phase, washed, resuspended in either (A) YPD + UFAs or (B) YPD, and incubated for 14.5 hr at 37° prior to electron microscopy. The nucleus (N) is indicated and magnification is ×20,000.

by supplementing the growth medium with UFAs, we determined whether *ole1* and *mga2*Δ *spt23-ts* mutants had similar phenotypes. We disrupted one copy of *OLE1* in an *OLE1*/*OLE1* diploid strain by single-step gene disruption using a restriction fragment containing *ole1::LEU2*. The correct disruption was verified by genomic Southern blot analysis (S. Zhang, unpublished results). Dissection of the resulting *ole1::LEU2*/*OLE1* diploid on medium supplemented with UFAs resulted in four viable spores in each of five tetrads. As expected, there was a 2:2 segregation for the UFA requirement and all UFA^- cells were Leu^+. To examine the cellular morphology of the *ole1* mutant after starvation for UFAs, strain SZ66 was grown to early log phase in YPD broth supplemented with UFAs and washed extensively to remove residual UFAs, as previously described by Stukey *et al.* (1989). The *ole1* mutant was resuspended in either supplemented or unsupplemented YPD media and incubated for 14.5 hr at 37° to further deplete UFAs from the unsupplemented cells. Cells treated in this way have UFA levels <10% of total cellular fatty acids (Stukey *et al.* 1989). Electron microscopy of the *ole1* mutant that was either supplemented (Figure 6A) or starved (Figure 6B) for UFAs revealed severe morphological defects when the mutant was depleted of UFAs. Many membrane-like structures separated by unstained material were apparent, but morphologically recognizable subcellular structures, such as mitochondria and nuclei, were not observed in these cells. UFA starvation for 6 hr or incubation at 30° resulted in similar morphological defects (S. Zhang, unpublished results). The severity of the *ole1* mutant phenotype suggests that UFA synthesis is not completely blocked in the *mga2*Δ *spt23-ts* mutant.

***GAL80* and *OLE1* transcript levels in the *mga2*Δ *spt23-ts* mutant:** To identify additional target genes activated by Mga2p/Spt23p, we performed RNA differential display followed by Northern blot analysis with RNA extracted from the wild type and the *mga2*Δ *spt23-ts* mutant grown at 37°. Candidate genes having a lower level of transcripts as well as differential display products included *CRY1*, *UBI2*, *YLR388w*, and *YMR142c* (S. Zhang, unpublished results). However, we were unable to reproducibly obtain the same reduction in candidate gene transcript levels from independent RNA preparations. These results also raised the concern that an Mga2p/Spt23p-independent RNA polymerase II transcript, which would be required as a loading control for further analysis of *OLE1* transcription, would be difficult to obtain. Therefore, we identified RNA differential display products that remained constant under all conditions. One of the strongest constant display products was from the *GAL80* gene. This result was verified by Northern blot analysis using independent RNA preparations (S. Zhang, unpublished results). Because the level of the *GAL80* transcript was not controlled by *MGA2* or *SPT23*, *GAL80* was used as an internal loading control to quantitate the relative level of the *OLE1* transcript.

We performed Northern blot analysis using total RNA from different strains to determine whether Mga2p/Spt23p are required for *OLE1* transcription (Figure 7). Similar levels of the *OLE1* transcript were produced in the wild-type strain (Figure 7A, lane 1) and *mga2*Δ (lane 2) or *spt23*Δ (lane 3) single mutant strains, compared with the level of the *GAL80* transcript. When the *mga2*Δ *spt23-ts* mutant was analyzed at 37°, however, very little *OLE1* transcript was detected (lane 4). Introduction of a multicopy plasmid carrying *OLE1* increased the *OLE1* transcript well above (lane 5) the wild-type level. Adding exogenous UFAs did not increase the level of *OLE1* transcript in the mutant at 37° (S. Zhang, unpublished results), minimizing the possibility that an alternative pathway mediates suppression of growth by UFAs and expression of *OLE1*. In addition, the transcript level from *ACC1*, another gene required for fatty acid biosynthesis (Figure 1), remained unaltered in the *mga2*Δ *spt23-ts* mutant (S. Zhang, unpublished results), suggesting that not all fatty acid biosynthetic genes require Mga2p/Spt23p.

To quantitate the level of the *OLE1* transcript in the absence of functional Mga2p/Spt23p, we performed Northern blot analysis with RNA extracted from the wild type and the *mga2*Δ *spt23-ts* mutant 0, 10, 30, and 60 min after shifting the cultures from 23° to 37° (Figure 7B). Phosphorimage analysis of the resulting filters indicated that the level of the *OLE1* transcript remained constant, compared with the *GAL80* transcript loading control throughout the time course in wild-type cells (lanes 1–4). The *OLE1* transcript level in the mutant (lane 5; 0 min) was about threefold lower than that in the wild type (lane 1; 0 min) at the time of the temperature shift. The lower level of the *OLE1* transcript in the *mga2*Δ *spt23-ts* mutant at 23° is probably due to a moderate loss of activity of the Spt23-ts protein at the permissive temperature. Shifting the mutant cells to 37° decreased the level of the *OLE1* transcript 15-fold after 10 min (lane 6), and 30-fold after 30 (lane 7) or 60 (lane 8) min, compared with the level of the *OLE1* transcript in the wild type at equivalent time points.

Suppression by *CEN-OLE1*: We considered two models that might explain the suppression of *mga2*Δ *spt23-ts* when *OLE1* is carried on a low-copy centromere plasmid. Mga2p/Spt23p may be required for centromere function, thereby allowing the plasmid copy number to increase significantly when Mga2p/Spt23p are absent. Alternatively, the amount of the *OLE1* transcript produced by the *mga2*Δ *spt23-ts* mutant may be just below the threshold required for growth, so that even a modest increase in the copy number of *OLE1* would allow cell growth. Therefore, we determined the relative copy number of the *URA3*-based centromere vector pRS416 and *CEN-OLE1* compared with the chromosomal *ura3-52* locus by Southern blot analysis in both wild-type and mutant cells at 23° and 37° (refer to materials and methods). A 1.5-fold increase in the copy number of

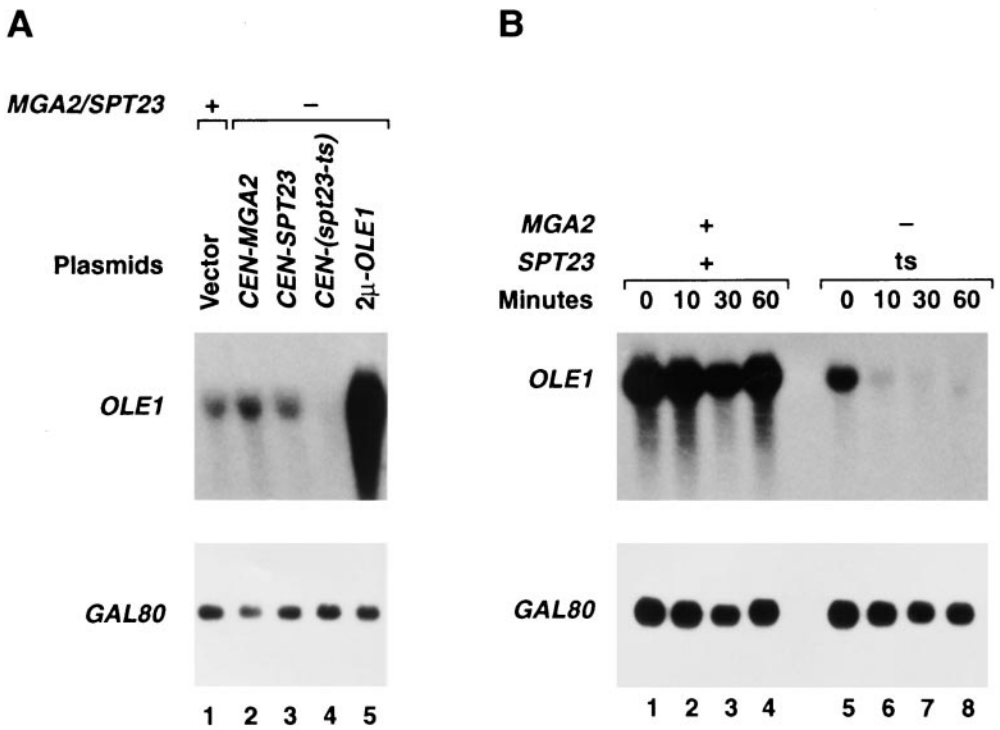

Figure 7.—Mga2p/Spt-23p affect the level of the *OLE1* transcript. (A) Total yeast RNA was prepared from strain DG1555 containing plasmid pRS426 (Vector, lane 1), SZ60 (*CEN-MGA2 spt23*Δ, lane 2), SZ30 (*CEN-SPT23 mga2*Δ, lane 3), DG1667 (*mga2*Δ *spt23-ts*, lane 4), and DG-1667 containing pSRZ157 (*mga2*Δ *spt23-ts*, 2μ-*OLE1*, lane 5). All the strains were pregrown at 23° to early log phase and shifted to 37° for 6 hr prior to RNA extraction. (B) Total RNA was prepared from wild-type strain DG1555 (lanes 1–4) and the *mga2*Δ *spt23-ts* mutant DG1667 (lanes 5–8) at the designated times after the cultures were shifted from 23° to 37°. In A and B, duplicate Northern blots containing 10 μg of RNA per sample were hybridized with ^{32}P-labeled DNA probes specific for *OLE1* and *GAL80* transcripts. Additional strain information is at the top.

pRS416 or *CEN-OLE1* was observed in the *mga2*Δ *spt23-ts* mutant at 37° (data not shown).

To determine the effect of *OLE1* copy number on *mga2*Δ *spt23-ts*, we directed the integration of the YIp plasmid pSRZ176, which contains the same *OLE1* insert as that in *CEN-OLE1* (pSRZ161), to the *ura3-52* locus of strain DG1667 by linearizing pSRZ176 with *Stu*I, which cleaves pSRZ176 once in the *URA3* gene. Ura$^+$ transformants containing one to four copies of pSRZ176 integrated at the *ura3-52* locus (refer to materials and methods) were analyzed for growth by endpoint dilution and for *OLE1* transcript level at 37° (Figure 8). Increasing the copy number of *OLE1* in the *mga2*Δ *spt23-ts* mutant cells improved cell growth (Figure 8A), indicating that the mutant is sensitive to *OLE1* gene dosage. Northern blot analysis revealed that the level of *OLE1* transcript is also sensitive to the copy number of *OLE1* in both the wild-type (Figure 8B, lanes 1 and 2) and the *mga2*Δ *spt23-ts* mutant (Figure 8B, lanes 3–7) strains. However, there was a discrepancy in the level of the *OLE1* transcript (Figure 8B, lane 8) and the degree of cell growth for the mutant cells carrying *CEN-OLE1* (Figure 8A, *CEN-OLE1*): these cells grew as well as a mutant integrant containing two additional copies of *OLE1*, but the level of the *OLE1* transcript contributed by *CEN-OLE1* was lower than expected. It is possible that a combination of small differences in the stability and copy number of the *CEN-OLE1* plasmid influence the suppression, because our data clearly show that the *OLE1* transcript level in *mga2*Δ *spt23-ts* cells is just below the threshold required for growth at the nonpermissive temperature.

DISCUSSION

Here we report the isolation of *SWI5* and *OLE1* as plasmid-borne suppressors of *mga2 spt23* lethality. Cells deficient in Mga2p/Spt23p have a lower level of UFAs and pronounced nuclear membrane alterations. These phenotypes are not as severe, however, as those observed in an *ole1* null mutant. Suppression of *mga2 spt23* or *ole1* lethality also occurs when the growth medium is supplemented with the enzymatic products of Ole1p, palmitoleic and oleic acids. Finally, the loss of function of both *MGA2* and *SPT23* results in at least a 15-fold decrease in the level of the *OLE1* transcript. Taken together, our results suggest that *MGA2* and *SPT23* are redundant activators required for full transcription of the essential metabolic gene *OLE1*.

In the absence of Mga2p/Spt23p, the expression of *OLE1* and the resulting synthesis of UFAs are below the threshold required for growth. Although the ratio of SFAs to UFAs is important for maintaining optimal membrane-associated enzyme activities and transport

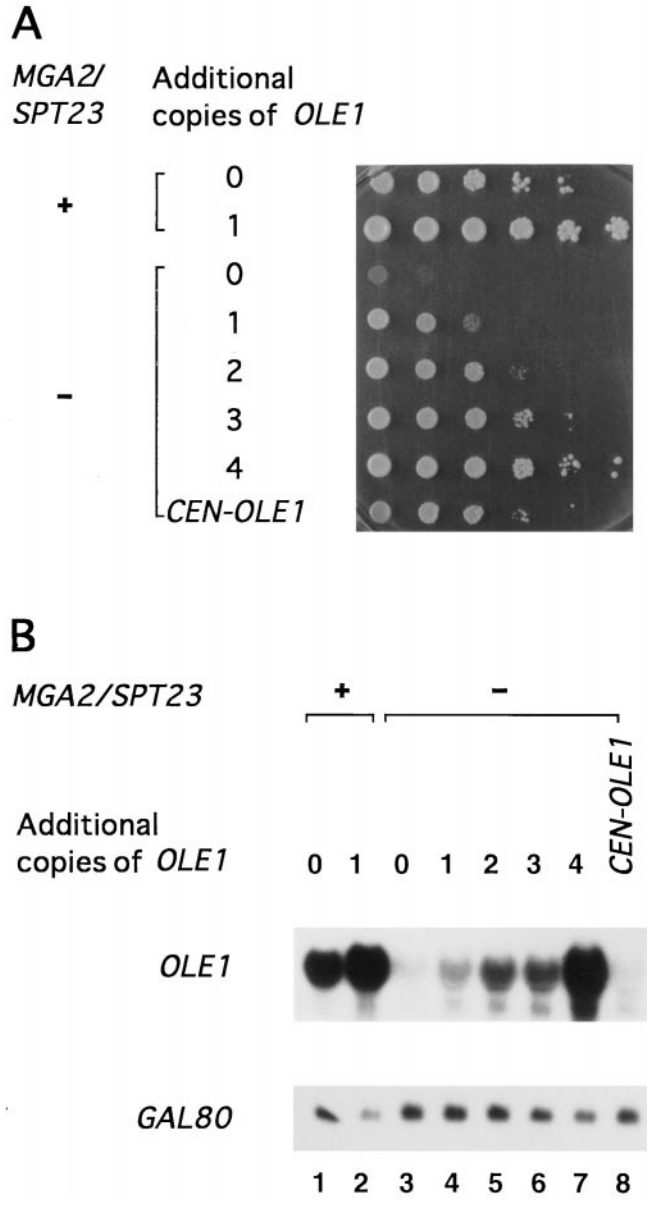

Figure 8.—Growth of *mga2*Δ *spt23-ts* mutant cells at 37° depends on *OLE1* gene dosage. (A) From the top to bottom, strains DG1555 (+, *MGA2 SPT23*) containing 0 or 1 additional copy of *OLE1* integrated at the *URA3* locus, DG1667 (−, *mga2*Δ *spt23-ts*) containing 0–4 additional copies of *OLE1* at *URA3*, and DG1667 containing pSRZ161 (*CEN-OLE1*) were grown in SC-Ura medium to midlog phase at 23°. Ten-fold serial dilutions of the cultures were spotted onto an SC-Ura plate from left to right, and incubated for 4 days at 37°. (B) Total RNA was prepared from liquid cultures of the above strains 6 hr after shifting the cultures from 23° to 37°. Duplicate Northern filters containing 10 μg of total RNA were prepared and hybridized with ^{32}P-labeled DNA probes specific for *OLE1* and *GAL80* transcripts.

processes (Krulwich *et al.* 1990; Schneiter and Kohlwein 1997), analyses of mutations affecting fatty acid biosynthesis have revealed complexities in membrane structure and function that are not completely understood. In particular, the *mdm2* mutation causes temperature-sensitive growth and failure to transfer mitochondria into the growing bud at the nonpermissive temperature and is an allele of *OLE1* (McConnell *et al.* 1990; Stewart and Yaffe 1991). The mitochondria fragments and aggregates in the mother cell and the UFA level comprises only 30% of total fatty acids. Electron microscopic analysis of the *mdm2* mutant, however, does not reveal any morphological alterations other than retention and fragmentation of mitochondria in the mother cells during mitotic growth. In contrast, UFAs are present at a higher level in the *mga2*Δ *spt23-ts* mutant than in *mdm2*, yet the nuclear envelope morphology is altered in the *mga2*Δ *spt23-ts* mutant. In addition, when rat liver stearoyl-CoA desaturase replaces Ole1p, the level of UFAs in these cells is slightly less than that observed when Mga2p/Spt23p are absent (42 *vs.* 48.1%, respectively), but nuclear membrane alterations are not observed (Stukey *et al.* 1990).

Another membrane-associated process that responds to a change in fatty acid content is exemplified by a mutation in *ACC1*, which encodes acetyl-CoA carboxylase (Figure 1). The *acc1-7-1* mutant was isolated in a screen for genes involved in mRNA transport to the cytoplasm (Kadowaki *et al.* 1994). Like *mga2*Δ *spt23-ts*, the *acc1-7-1* mutation causes nuclear membrane separation and the appearance of vesicle-like structures in the intermembrane space (Schneiter *et al.* 1996). The *acc1-7-1* mutant fatty acid profile shows a remarkable reduction in the level of very long chain fatty acids, such as C26, but not in the levels of other fatty acids. We have not determined the level of very long chain fatty acids in the *mga2*Δ *spt23-ts* mutant; therefore, a reduction in the C26 level may also contribute to the nuclear membrane defect observed in our work. However, three results suggest that the *mga2 spt23* and *acc1-7-1* mutations act differently. First, our results suggest that the transcript levels from several genes decrease in the *mga2*Δ *spt23-ts* mutant, instead of being retained in the nucleus. Second, unlike *acc1-7-1*, both the lethality and the morphological defects in the *mga2*Δ *spt23-ts* mutant are reversed by supplementation with UFAs. Third, Mga2p/Spt23p are not required for *ACC1* transcription.

Because the lethality and the morphological defects observed in the absence of Mga2p/Spt23p are reversed by supplementation with UFAs, these phenotypes are probably caused by a deficiency in UFA synthesis. Results from our work, as well as the results from others mentioned above, show that correlating UFA levels with morphological alterations and cell viability is not straightforward and suggest that other lesions in the *mga2*Δ *spt23-ts* mutant contribute to the morphological alterations in the nuclear membrane. Therefore, identifying other cellular processes influenced by *MGA2* and *SPT23* will be necessary to completely explain their roles in the cell. An attractive starting point for these studies is to understand the molecular basis of *mga2*Δ *spt23-ts*

suppression by the gene-specific transcriptional activator Swi5p.

Our results implicate *OLE1* as the essential target gene whose expression is dependent on Mga2p/Spt23p. Mga2p/Spt23p probably affect *OLE1* transcription, although we cannot exclude the possibility that Mga2p/Spt23p influence *OLE1* mRNA stability. Mga2p/Spt23p do not affect general mRNA stability, however, because *GAL1* mRNA decayed at the same rate when wild-type or *mga2*Δ *spt23-ts* cells were shifted from galactose to a repressing carbon source, glucose, at 23° or 37° (S. Zhang, unpublished results). If *MGA2*/*SPT23* stimulate transcription of *OLE1* and Ty1, and probably other genes, how does this happen? No known DNA-binding motif has been found in either gene. We have proposed that *MGA2*/*SPT23* encode related transcriptional coactivators that may act by changing chromatin accessibility (Zhang *et al.* 1997). These proteins may also interact with sequence-specific DNA binding proteins. Interestingly, the promoter region of the *Histoplasma capsulatum OLE1* gene (*OLE1-Hc*) has been analyzed by deletion, gel mobility shift, and DNase footprinting analyses (Tosco *et al.* 1997). An AP1 binding site (TGACTAA) 740 bp upstream of the initiator ATG is required for *OLE1-Hc* transcription and binds nuclear proteins from the yeast and mycelial phases of *H. capsulatum.* The *S. cerevisiae* and *H. capsulatum OLE1* genes encode virtually identical proteins that are also closely related to higher eukaryotic Δ9 fatty acid desaturases (Stukey *et al.* 1990; Anamnart *et al.* 1997; Gyorfy *et al.* 1997). Therefore, we scanned the sequence 900 bp upstream of *OLE1* (*OLE1-Sc*) for potential AP1 binding sites. The *OLE1-Sc* region contains one putative AP1 binding site (TGACTAT) at position −636. The *OLE1-Sc* AP1 site is contained within the subclones used in our analyses, and deletions encompassing this sequence lower the level of *OLE1-Sc* transcripts (Choi *et al.* 1996). Furthermore, there are eight yeast AP-1-like bZIP proteins identified by sequence and mutational analyses, in addition to the well-studied AP-1/bZIP transcriptional activator Gcn4p. Fernandes *et al.* (1997) have shown that several of the yeast AP-1 factors (designated Yap proteins) are transcriptional activators with distinct biological functions. We are currently investigating whether Mga2p/Spt23p act as coactivators in concert with Yap proteins and the Snf/Swi chromatin remodeling complex to stimulate *OLE1* transcription through its AP1 binding site.

Our studies suggest that *GAL80* has unique features, because its transcription appears to be independent of Mga2p/Spt23p. *GAL80* is, in fact, unusual in that two pathways are used to initiate transcription (Sakurai *et al.* 1994). The first is constitutive, stimulated by a specific upstream activating sequence (UAS), independent of the TATA box, and transcription initiates at a specific start site called +1. The second pathway is induced by galactose and repressed by glucose and, under the control of the Gal4 activator protein and a UAS_{GAL} binding site, depends on the TATA box and initiates transcription at several sites downstream of +1. Because all the RNA samples used in our analyses are from cells grown in media containing glucose, the *GAL80* constitutive transcript is not under Mga2p/Spt23p control. Whether Mga2p/Spt23p-independence is characteristic of all TATA-independent transcription units or involves specific upstream activating sequences remains to be determined.

In summary, our continued investigation of *MGA2* and *SPT23* illustrates the power of using suppression of Ty-induced mutations to identify components important for gene expression (Winston 1992). We have now linked a fatty acid and Ty gene expression through the identification of *OLE1* as a target gene for *MGA2*/*SPT23*. This result is consistent with the idea that Ty elements and the yeast genome have coevolved such that the cell can modulate the level of Ty gene expression and hence transposition, through multiple regulatory pathways (Boeke and Sandmeyer 1991; Garfinkel 1992).

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