**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, SW15, and the Δ9 fatty acid desaturase of yeast, OLE1, as multicopy suppressors of an mga2Δ spt23 temperature-sensitive mutation (spt23-ts). The level of unsaturated fatty acids decreases 35–40% when the mga2Δ spt23-ts mutant is incubated at 37°C. 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 mga2Δ spt23-ts and mga2Δ spt23Δ 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 (Suppressor of Ty) genes. These include genes encoding the TATA-binding protein TBP (SPT15; Eisenmann et al. 1989), histone core proteins H2A and H2B (SPT11 and SPT12; Clarke and Burkett 1992; and Strittmatter and Winston 1989), histone chaperones H2A and H2B (SPT11 and SPT12; Ciar-A-Dams 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 snf2Δ 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 mga2Δ 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; Schneider et al. 1996; Schneider and Kohlein 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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acetyl-CoA
Acc1p

malonyl-CoA
free fatty acids

\[ \text{[Fas1p] + Elo1p} \]
\[ \text{Faa1p-Faa4p} \]

\begin{align*}
\text{palmitoyl-CoA (16:0)} \\
\text{stearoyl-CoA (18:0)} \\
\text{Ole1p}
\end{align*}

\begin{align*}
\text{palmitoleic acid (16:1)} \\
\text{oleic acid (18:1)} \\
\text{very long chain fatty acids (C20-C30)}
\end{align*}

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 (Bossize 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, dis-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 acetyl-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 mga2Δ spt23-ts mutant at the nonpermissive temperature or a mga2Δ spt23Δ double mutant. Electron microscopy shows that the mga2Δ 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 Sad-HindIII 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 site in URA3. Strain SZ67 was obtained by tetrad dissection of a diploid strain heterozygous for spt23ΔhisG and mga2Δ::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 Stucky 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, IN), 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 hydroxyamine (Zhang et al. 1997). Plasmids pSMS1, pSMS6, pSMS7, pSMS8, pSM9, and pSMS22 were isolated as suppressors of the lethality conferred by mga2Δ spt23-ts at 37°. Plasmids pSRZ157 (2μ::OLE1), pSRZ161 (CEN-OLE1), and pSRZ176 were constructed by sub-cloning a 3.1-kb Sad-HindIII fragment containing OLE1 from pSMS7 into pRS426, pRS416, and pRS406 (Sikorski and Hieter 1989), respectively. Plasmid pSRZ159 (2μ::ole1) was constructed by digesting pSRZ157 with Tth111I, 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 HindIII. Total yeast DNA from certain transformants was isolated after cells were shifted to 37° for 6 hr. The resulting filter was hybridized with a 32P-labeled internal Nde-Stul fragment from URA3. The transformants contained two types of HindIII frag-
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 HindIII 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 EcoRV digestion. The resulting filter was hybridized with a 32P-labeled URA3 probe as described above. The integrants contained two EcoRV 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; Katay 1986). Briefly, yeast cells were broken by vortexing in the presence of glass beads in methanol:chloroform:water (2:1:0.8). Total lipids were saponified with sodium hydroxide, then esterified by transmethylation with boron trifluoride at 80°. Residual DNA was removed using the Megaprime DNA Labeling System (Amersham, Buckinghamshire, UK). The template for labeling ole1 was a 1.3-kb Sall-Pad fragment of pSRZ175. The template for the GAL80 probe was a 1.3-kb genomic fragment amplified by PCR using primers 5'-CCACTCCCCTCATGGAC-3' and 5'-GGGGCAAGCACAG-3'. The template for the ACC1 probe was a 1.9-kb genomic fragment amplified by PCR using primers 5'-CCCGACAGGCTTC-3' and 5'-GTACCCTCCTCACAG-3'. Hybridization signals were quantitated by phosphorimaging 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.

**RESULTS**

***Isolation of plasmid-borne suppressors of mga2Δ***: The synthetic lethality of an mga2Δ spt23-ts 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 mga2Δ 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
OLE1 results), because a subclone containing only the S. Zhang, mga2 (2 
we reisolated Genome Data Base (Stanford University). As expected, gene and of Saccharomyces borne suppressors was submitted to the sequence from the genomic inserts of the six plasmid- published results). The suppression by the other 14 isolates probably resulted from chromosomal mutations; we will characterize these suppressors elsewhere. by DNA sequencing and mutagenesis indicated that the suppressor and Spt23p. Analysis of its genomic insert 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 (pSM58 and pSM59; in Figure 2, pSM58 is shown) and SPT23 once (pSM6). Strain DG1667 containing pSM6, pSM8, or pSM59 grew well at 37°, compared with the DG1667 (vector) mutant and DG1555 (Wt./vector) wild-type control strains. The suppressor plasmids pSM57 and pSMS22 contained overlapping inserts, as demonstrated by DNA sequence and restriction enzyme analyses (S. Zhang, unpublished results). The suppression by pSM57 and pSM22 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 pSM57 and pSMS22 also showed that these plasmids suppressed lethality in an mga2Δ spt23Δ mutant. Deletion and site-directed mutagenesis of the insert in pSM57 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 mga2Δ spt23-ts (2μ-OLE1) and a frameshift mutation in the OLE1 cod-
TABLE 2

<table>
<thead>
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<td>37°</td>
<td>15</td>
<td>11.3</td>
</tr>
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</table>

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

Suppressors of mga2 spt23 lethality

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 mga2Δ 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 mga2Δ 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 (mga2Δ 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 mga2Δ spt23-ts and the unconditional lethality of the mga2Δ spt23Δ 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 mga2Δ 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 mga2Δ spt23Δ 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 (mga2Δ spt23-ts), and (C) SZ67 (mga2Δ 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 (OD600nm). Each measurement was the average from three experiments, and the doubling time for a given strain in each experiment was comparable (±15%).
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 mga2Δ spt23-ts mutant (Figure 5) and an ole1 null mutant (Figure 6) by electron microscopy. The mga2Δ 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 mga2Δ spt23-ts mutant was severely distorted at 37° (Figure 5B). Like acc1-7-1, the mga2Δ 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 mga2Δ spt23-ts mutant (Figure 5C).

Because the cell growth and morphological defects present in the spt23 mga2 double mutants are reversed
by supplementing the growth medium with UFAs, we determined whether ole and mga2Δ 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 ole::LEU2. The correct disruption was verified by genomic Southern blot analysis (S. Zhang, unpublished results). Dissection of the resulting ole::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 transcript well above (lane 5) the wild-type level. Adding four viable spores in each of five tetrads. As expected, UFA starvation for 6 hr or incubation at 30° resulted in similar morphological defects (S. Zhang, unpublished results). The severity of the ole mutant phenotype suggests that UFA synthesis is not completely blocked in the mga2Δ spt23-ts mutant.

GAL80 and OLE1 transcript levels in the mga2Δ 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 mga2Δ 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 YM R142c (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 quantify 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 mga2Δ (lane 2) or spt2Δ (lane 3) single mutant strains, compared with the level of the GAL80 transcript. When the mga2Δ 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 mga2Δ 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 mga2Δ 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 mga2Δ spt23-ts mutant at 23° is probably due to a moderate loss of activity of the Spt23-ts protein at the permissive temperature. Shifting 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 mga2Δ 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 mga2Δ 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 spt23Δ, lane 2), SZ30 (CEN-SPT23 mga2Δ, lane 3), DG1667 (mga2Δ spt23-ts, lane 4), and DG1667 containing pSRZ157 (mga2Δ 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 mga2Δ 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 32P-labeled DNA probes specific for OLE1 and GAL80 transcripts. Additional strain information is at the top.

Discussion

Here we report the isolation of SWI5 and OLE1 as plasmid-born suppressors of mga2Δ spt23 lethality. Cells deficient in Mga2p/Spt23p have a lower level of UFA synthesis and pronounced nuclear membrane alterations. These phenotypes are not as severe, however, as those observed in an ole1 null mutant. Suppression of mga2Δ spt23 or ole1 lethality also occurs when the growth medium is supplemented with 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
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mga2 spt23

Lethality
ture-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, UFA levels are present at a higher level in the mga2Δ spt23-ts mutant than in mdm2, yet the nuclear envelope morphology is altered in the mga2Δ 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 (Stuekey 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 mga2Δ 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 mga2Δ 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 mga2Δ spt23-ts mutant, instead of being retained in the nucleus. Second, unlike acc1-7-1, both the lethality and the morphological defects in the mga2Δ 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 mga2Δ 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 mga2Δ 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 mgaΔ spt23-ts cells were shifted from galactose to a repressing carbon source, glucose, at 23°C or 37°C (S. Zhang, unpublished results). If MGA2/SPT23 stimulate transcription of OLE1 and YTL, 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 co-activators 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-H) 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-H transcription and binds nuclear proteins from the yeast and mycelial phases of H. capsulatum. The S. cerevisiae 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; Gyory 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 (Winist 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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