Regulation of Unsaturated Fatty Acid Biosynthesis in Saccharomyces

THE ENDOPLASMIC RETICULUM MEMBRANE PROTEIN, Mga2p, A TRANSCRIPTION ACTIVATOR OF THE OLE1 GENE, REGULATES THE STABILITY OF THE OLE1 mRNA THROUGH EXOSOME-MEDIATED MECHANISMS*‡

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The Saccharomyces cerevisiae OLE1 gene encodes a membrane-bound Δ9 fatty-acid desaturase, whose expression is regulated through transcriptional and mRNA stability controls. In wild type cells grown on fatty acid-free medium, OLE1 mRNA has a half-life of 10 ± 1.5 min (basal stability) that becomes highly unstable when cells are exposed to unsaturated fatty acids (regulated stability). Activation of OLE1 transcription is dependent on N-terminal fragments of two membrane proteins, Mga2p and Spt23p, that are proteolytically released from the membrane by a ubiquitin-mediated mechanism. Surprisingly, disruption of the MGA2 gene also reduces the half-life of the OLE1 transcript and abolishes fatty acid regulated instability. Disruption of its cognate, SPT23, has no effect on the half-life of the mRNA. Mga2p appears to have two distinct functions with respect to the OLE1 mRNA stability: a stabilizing effect in cells grown in fatty acid-free medium and a destabilizing function in cells that are exposed to unsaturated fatty acids. These functions are independent of OLE1 transcription and can confer basal and regulated stability on OLE1 mRNAs that are produced under the control of the unrelated GAL1 promoter. Expression of soluble, N-terminal fragments of Mga2p stabilize the transcript but do not confer fatty acid-regulated instability on the mRNA suggesting that the stabilizing functions of Mga2p do not require membrane processing and that modifications to the protein introduced during proteolysis may play a role in the destabilizing effect. An analysis of mutants that are defective in mRNA degradation indicate that the Mga2p-requiring control mechanism that regulates the fatty acid-mediated instability of the OLE1 transcript acts by activating exosomal 3′ → 5′-exonuclease degradation activity.

Unsaturated fatty acids are formed in the yeast Saccharomyces cerevisiae by Ole1p, a membrane-bound Δ9 fatty-acid desaturase that converts long chain saturated fatty acyl-CoA substrates into monounsaturated species (1, 2). Expression of OLE1 is regulated by nutrient fatty acids by both transcriptional and mRNA stability controls. Recently, the expression of Ole1p was found to be dependent on two homologous ER membrane proteins, Mga2p and Spt23p (3). These are cleaved from the membrane by a novel ubiquitin-mediated proteolytic mechanism that releases soluble N-terminal polypeptides (4). Although neither protein appears to have a DNA binding domain, the N-terminal fragments specifically activate OLE1 transcription presumably by influencing chromatin structure or by associating with DNA binding proteins that are bound to elements on the OLE1 promoter (5, 6). Disruption of either SPT23 or MGA2 does not affect the growth rate or production of fatty acids under normal laboratory growth conditions (7). Disruption of both genes, however, blocks expression of Ole1p and results in a synthetic auxotrophy that can be overcome by adding unsaturated fatty acids to the medium or expressing OLE1 under a different promoter (4). Evidence for the direct role of Mga2p in OLE1 transcription has been provided in studies by Jiang et al. (5), who demonstrated that under conditions where OLE1 expression is induced by oxygen starvation, Mga2p is associated with the low oxygen response element in the OLE1 promoter.

In this study we show that, in addition to its role in the activation of OLE1 transcription, Mga2p but not Spt23p is linked to mechanisms that stabilize the OLE1 mRNA under fatty acid-free growth conditions and is essential for its regulated instability in cells exposed to unsaturated acids via mechanisms that require 3′ → 5′-exosomal mRNA degradation activity. Furthermore, whereas expression of soluble, N-terminal fragments of Mga2p stabilize the OLE1 transcript, those mRNAs are not destabilized by unsaturated fatty acids suggesting that post-translational modifications associated with the proteolytic processing of Mga2p may play a role in fatty acid-regulated mRNA stability.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—S. cerevisiae strains used in this study are shown in Table I. E. coli strains XL-BLUE (Stratagene, La Jolla, CA) and DH5α (Invitrogen) were used for DNA cloning and propagation of plasmids. E. coli cells were grown according to standard protocols (8, 9). Yeast cells were grown as described previously (10). The cells were

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1 The abbreviations used are: OLE1, gene encoding Ole1p, a Δ9 fatty acid desaturase; mRNP, messenger RNA ribonucleoprotein; ER, endoplasmic reticulum; SPT23, gene encoding the ER membrane Spt23p protein; spt23Δ, null (gene disrupted) allele of the SPT23 gene; MGA2, gene encoding the ER membrane Mga2p protein; mga2Δ, null (gene disrupted) allele of the MGA2 gene.
grown at 30 °C in complete synthetic medium + 1% Tergitol Nonidet P-40 (Sigma) lacking appropriate amino acids (based on the selectable marker used in the incorporated plasmid). Typically, the carbon source was 2% glucose. However, when the cells contained the gene of interest fused to a galactose-inducible promoter, they were grown overnight in medium containing 2% raffinose. 2% galactose or 2% glucose was added to the growth medium when required to induce or repress transcription of genes under the control of the GAL1 promoter. Ununsaturated fatty acids (obtained from Sigma) were added to the growth medium to a final concentration of 15 μg/ml.

DNA Isolation and Manipulations—Methods for general DNA manipulations were performed according to standard procedures described in Ausubel et al. (6) and Maniatis et al. (9). Yeast transformations were performed using the YEASTMAKER yeast transformation system (Clontech Laboratories, catalog no. K1606-1) following the manufacturer's directions.

Plasmid Constructions—Plasmids used in this study are described in Table I. Those plasmids that express C-terminal deleted forms of Mga2p were made by PCR amplification of N-terminal regions of the gene followed by splicing the amplified fragment into the appropriate vector according to standard protocols.

mRNA Decay Measurements—mRNA decay rates were determined by rapidly arresting transcription of OLE1 and monitoring the decay of its mRNA levels as described previously (10). Transcription of the native OLE1 gene was arrested by using the transcriptional inhibitor thiolutin (15 μg/ml). Measurements of the half-life of the OLE1 mRNA produced under control of the yeast GAL1 promoter were conducted by initially growing cells on raffinose as a carbon source. Transcription of the OLE1 mRNA was induced by the addition of 2% galactose and then arrested by adding 2% glucose. RNA isolations and quantitative Northern blot analysis of cells following transcription arrest were performed as described previously (10).

Preparation of Radiolabeled Probes—For the detection of OLE1 mRNA, a radiolabeled DNA probe was made using a 1.0-kb EcoRI fragment from the OLE1 coding sequence isolated from YEp352-OLE4.8. For all experiments, the internal control mRNA was detected with a 1.0-kb HindIII-KpnI radiolabeled fragment of the phosphoglycerate kinase (PGK1) gene isolated (11) or an 800-bp PCR amplified fragment of the yeast actin gene (ACT1). All DNA fragments were separated by agarose gel electrophoresis in 1× Tris acetate-EDTA buffer and purified by Gene Clean II (Bio 101). The DNA fragments were labeled to high specific activity with [α-32P]dATP (PerkinElmer Life Sciences) by the random primer extension method using the NEBlot kit from New England Biolabs. Unincorporated nucleotides were removed from the sample using a Sephadex G-50 spin column. The specific activities of labeled probes were determined by liquid scintillation counting.

Analyses of Data—Statistical analyses were performed with SigmaPlot software. Statistical significance was determined by performing the Student's t test. p values <0.05 were taken as a significant difference.

RESULTS

Mga2p but Not Spt23p Is Required for the Fatty Acid-Mediated Regulation of OLE1 mRNA Stability—Mga2p and Spt23p are two homologous proteins that are required for the transcriptional activation and expression of the OLE1 gene (3, 4). To test the individual effects of Spt23p- and Mga2p-induced expression on OLE1 mRNA stability, the decay kinetics of the native transcript were determined in cells containing a disrupted form of each gene (Fig. 1). Cells that contained a functional MGA2 gene and a disrupted SPT23 gene (spt23Δ/ΔMGA2) exhibited the normal pattern of basal and regulated OLE1 mRNA decay with an average half-life of 10.5 min when grown in fatty acid-depleted medium and 4.5 min after exposure to fatty acids. By contrast, cells that contain the wild type SPT23 gene and a disrupted MGA2 gene (spt23Δ/mga2Δ) showed identical half-lives of 6 min under fatty acid-deprived and fatty acid-exposed conditions, indicating that the basal stability and regulated instability was lost. These data indicate that Mga2p acts in this regulation system in two ways: to stabilize the OLE1 transcript in cells grown on fatty acid-depleted medium and to destabilize it when cells are exposed to unsaturated fatty acids. This pattern of behavior might occur either through a direct physical association of Mga2p with the OLE1 transcript or indirectly by the action of Mga2p through some regulatory pathway that alters the nature of the mRNA decay.

OLE1 transcription is activated through two promoter regions designated as the FAR and LORE elements (5, 12–14). Although Jiang et al. (5) demonstrated that Mga2p associates with the LORE element under hypoxic induction conditions, it is not clear whether Spt23p and/or Mga2p associate with those regions under other growth conditions. To determine whether the effects of Spt23p or Mga2p on mRNA stability of OLE1 require the activation of the transcription of its mRNA through these or other elements in the OLE1 promoter, we tested the stability of the OLE1 transcript produced under the control of the unrelated GAL1 promoter sequences (Fig. 2). We have shown previously that in wild type cells the half-life of OLE1 mRNA expressed under the control of the GAL1 promoter is similar to that of the native transcript, yielding a basal half-life of 10–12 min and a regulated half-life of <2 min (10, 15).

In experiments where OLE1 was expressed under the control of GAL1 promoter in an mga2Δ/spt23Δ strain (Fig. 2a), the half-life of the transcript in the both absence and presence of fatty acids was 6 min, which correlates with the loss of basal and regulated stability exhibited by the native transcript in cells lacking Mga2p. By contrast, in spt23Δ/mga2Δ cells, the average half-life of the GAL1 expressed transcript was 11 min in fatty acid-free medium and 4.5 min in cells exposed to 18:2 (Fig. 2b). These data indicate that the effects of Mga2p on the basal and regulated decay of OLE1 mRNA occur independently of its effects on OLE1 transcription activation through elements in the OLE1 promoter.

To test whether Spt23p contributed to overall OLE1 mRNA stability in the absence of Mga2p, we also used the GAL1 expressed OLE1 mRNA to measure its rate of decay in a strain containing disrupted forms of both genes. Under both conditions, the half-life of the transcript produced by plasmid pGAL-OLE2.8 was similar to those measured in the mga2Δ strains (Fig. 2c). Thus, even though Spt23p has substantial sequence and structural homology Mga2p, it does not appear to contribute to the basal or fatty acid-mediated regulation of OLE1 mRNA stability.

Expression of Soluble, N-terminal Fragments of Mga2p Stabilize OLE1 mRNA—Fatty acid-mediated instability of the transcript, however, requires expression of the full-length, membrane-bound form of the protein. Spt23p and Mga2p are intrinsic ER membrane proteins that are integrated into the

### Table I

| Strain | Genotype | Source |
|--------|----------|--------|
| mga2Δ::LEU2 | MATa, mga2Δ::LEU2, ura3-1, his3-11, his3-15, ade2-1, can1-100 | This laboratory |
| spt23Δ::LEU2 | MATa, spt23Δ::LEU2, ura3-1, his3-11, his3-15, ade2-1, can1-100 | This laboratory |
| mga2Δ::LEU2 | MATa, mga2Δ::LEU2, spt23Δ::LEU2, ura3-1, his3-11, trp1-1, ade2-1, can1-100 | This laboratory |
| spt23Δ::LEU2 | MATa, spt23Δ::LEU2, ura3-1, his3-11, trp1-1, ade2-1, can1-100 | This laboratory |
| ccr4–4 | MATa, trp1, ura3-1, leu2, his4, cap1::LEU2PM ccr4::NEO | D. Muhlrad, R. Parker |
| upf1-1 | MATa, leu2, his2, trp1, lys2, ura3, upf1::URA3 | D. Muhlrad, R. Parker |
| shi4–1 | MATa, trp1, leu2, ura3, his4, cap1::LEU2PM shi4–1 | D. Muhlrad, R. Parker |
membrane through a C-terminal membrane spanning domain (4, 7). Both proteins are cleaved by a novel ubiquitin-mediated mechanism that releases soluble N-terminal fragments from the membrane (4). These translocate to the nucleus and activate OLE1 expression. To determine whether the proteolytic processing of the membrane-bound Mga2p is required for regulated OLE1 mRNA stability, we tested the effects of two soluble forms of Mga2p with C-terminal deletions that are expressed under the control of their native promoter (Fig. 3A). Plasmid p87 expresses an approximate 87-kDa soluble Mga2p.

TABLE II

| Plasmid   | Description                                                                 | Source          |
|-----------|-----------------------------------------------------------------------------|-----------------|
| pGAL OLE2.8 | Contains an HpaI fragment of OLE1 gene containing 220 bases of 5’-UTR, the entire protein coding sequence and 1 kb of 3’-UTR under the control of yeast GAL1 promoter | This laboratory |
| p87       | Contains an N-terminal region of the Mga2p protein coding sequence that expresses an 87-kDa polypeptide under the control of its native promoter | This laboratory |
| p103      | Contains the entire Mga2p coding sequence under control of its native promoter. A stop codon was introduced near the C-terminus so that the expressed protein consists of a soluble, 103-kDa polypeptide | Dr. Alan Jiang |

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Fig. 1. Decay kinetics of native OLE1 mRNA in mga2Δ;SPT23 and spt23Δ;MGA2 strains. a and b, upper panels show phosphorimages of OLE1 and PGK1 mRNAs transcribed from their native promoters following inhibition of transcription by thiolutin. These were derived from Northern blots of total RNA isolated from strains containing deletions of MGA2 (a) or SPT23 (b) grown in medium containing either no fatty acids or 1 mM 18:2. The solid lines in a show the decay kinetics of OLE1 mRNAs in the corresponding mutant strains (●, no fatty acids; ○, 1 mM 18:2). The dashed lines in the graph in a show the decay kinetics of the OLE1 transcript in wild type cells grown under the same conditions (●, no fatty acids; ○, 1 mM 18:2, t1/2(avg) < 2 min). Phosphorimaging data was normalized using the PGK1 gene (t1/2 = 45 min) as an internal control. Error bars represent standard deviations derived from a minimum of three experiments for each mutant strain. The decay rates for the wild type strain are derived from the averages of six independent experiments. The lines through each set of data points represent a third order linear regression where r² > 0.95.

Fig. 2. Decay of OLE1 transcript expressed under the control of the GAL1 promoter in SPT23;mgα2Δ, spt23Δ;MGA2, and spt23Δ;mgα2Δ strains. Transcription of the OLE1 mRNA was induced with 2% galactose and arrested with 2% glucose. Half-lives shown to the right of the figures are averages of combined data from two independent experiments normalized using PGK1 as the internal control.

Half-life (minutes)

| Time (min) after transcription arrest | 1 mM 18:2 | + | - |
|--------------------------------------|-----------|---|---|
| a) mga2Δ; SPT23                     |           |   |   |
| pGAL-OLE2.8                          |           |   |   |
| OLE1                                 |           |   |   |
| PGK1                                 |           |   |   |
| Wild type                            |           |   |   |
| 6                                    | 6         | 6 |
| b) spt23Δ; MGA2                      |           |   |   |
| pGAL-OLE2.8                          |           |   |   |
| OLE1                                 |           |   |   |
| PGK1                                 |           |   |   |
| 11                                   | 4.5       |   |
| c) mga2Δ; spt23Δ                     |           |   |   |
| pGAL-OLE2.8                          |           |   |   |
| OLE1                                 |           |   |   |
| PGK1                                 |           |   |   |
| 5                                    | 5         | 5 |
Mechanisms of Regulated OLE1 mRNA Decay—We attempted to examine the mechanisms under which Mga2p contributes to the stability and regulation of the OLE1 transcript. Previous studies in this laboratory suggested that the primary basal decay mechanism of OLE1 has features associated with the general mRNA decay pathway (16–18), which requires deadenylaton-dependent decapping followed by degradation of the transcript by the 5' → 3' exonuclease, Xrn1p. A null mutation of the decapping enzyme, Dcp1p, makes the OLE1 transcript very stable under both basal and regulated conditions ($t_{1/2} > 50$ min to $>50$ min), indicating that decapping of the transcript is an obligate feature of the OLE1 regulation system (15). Similarly, a null mutation in Xrn1p stabilizes the transcript under basal conditions ($t_{1/2} = 45$ min). In those cells, however, the transcript is still destabilized by unsaturated fatty acids ($t_{1/2} = 23$ min) indicating that fatty acid-regulated decay proceeds via degradation mechanisms that are distinct from the general pathway (10).

Given that deadenylation of a number of mRNAs is required for the decapping reaction (16–18), we tested the effects of the loss of Ccr4p, the primary cytoplasmic deadenylase in yeast, to determine whether the fatty acid-regulated instability is a deadenylation-dependent function (Fig. 5). As expected, under basal conditions the reduced deadenylation activity in those cells stabilized the transcript approximately 2-fold, increasing the basal half-life from 10 to 20 min. Exposing the cells to unsaturated fatty acids also resulted in a longer regulated half-life (7 min) relative to wild-type cells. The net effect of stimulation by the fatty acids, however, was to strongly destabilize the transcript, inducing a net 3-fold increase in the mRNA degradation rate over basal conditions. These results indicate that Ccr4p-mediated deadenylation activity is not required to trigger fatty acid-regulated instability.

An alternative, nonsense-mediated decay pathway in yeast and other eukaryotes is associated with the decay of aberrant transcripts that contain premature stop codons and other features that prevent normal translation (19). We showed previously by introducing stop codons at different positions within the protein coding sequence that the OLE1 transcript is unusually resistant to this pathway (15). To test whether the downstream components of that regulation system might be required for the regulated decay of the normal OLE1 transcript, however, we examined the half-lives of OLE1 mRNA in cells containing a null mutation of the UPF1 gene (Fig. 5), which blocks nonsense-mediated decay. Deletion of Upf1p did not significantly change the OLE1 degradation rate under basal conditions ($p$ value = 0.82) but did produce a 2-fold increase in the fatty acid-regulated half-life (from 2.5 min to 5 min). Furthermore, the OLE1 transcript clearly exhibits a strong fatty acid-regulated response in upf1Δ cells (Fig. 5), indicating that the nonsense-mediated decay pathway is not required to induce fatty acid-regulated instability.

The finding that regulated decay of OLE1 mRNA requires decapping, but does not require the Xrn1p 5' → 3' exonuclease suggested that the regulated instability of OLE1 may involve the activation of exosomal 3' → 5' exonuclease activities. Exosomes are complex protein particles that play multiple roles in the processing and degradation of small RNAs (including the 3' trimming of 5.8 S rRNA and U4 small nuclear RNA precursors) in the nucleus and the 3' → 5' degradation of mRNAs in the cytoplasm (20, 21). Ski4p is one of 10 essential protein components of the core exosome particle, and a deletion of the entire SKI4 protein sequence is lethal. Cells that contain the ski4Δ−
allele, however, are viable because they have a point mutation in the RNA binding domain of the protein that inhibits the 3' → 5' degradation of cytoplasmic mRNAs while retaining the essential exosomal functions that are required for the degradation and processing of small nuclear RNAs (21).

We were surprised to find that in the ski4Δ cells the native OLE1 mRNA decay in an mga2Δspt25Δ strain expressing a soluble, 87-kDa or 103-kDa N-terminal fragment of Mga2p under the control of its native promoter. The OLE1 transcript is expressed under the control of its native promoter in cells grown on glucose. Transcription of OLE1 was arrested with the addition of thiolutin as described in Fig. 1. For the cells expressing the p87 form of Mga2p, the actin gene mRNA (ACT1) was used as the internal control.

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**FIG. 4.** OLE1 mRNA decay in an mga2Δspt25Δ strain expressing a soluble, 87-kDa or 103-kDa N-terminal fragment of Mga2p under the control of its native promoter. The OLE1 transcript is expressed under the control of its native promoter in cells grown on glucose. Transcription of OLE1 was arrested with the addition of thiolutin as described in Fig. 1. For the cells expressing the p87 form of Mga2p, the actin gene mRNA (ACT1) was used as the internal control.

**FIG. 5.** The OLE1 mRNA exhibits fatty acid-regulated instability in strains defective in deadenylation and nonsense mediated decay. The OLE1 transcripts in the figure are expressed under the control of its native promoter in either ccr4Δ or upf1Δ cells grown on glucose. Transcription of OLE1 was arrested with the addition of thiolutin in fatty acid-treated (UFA) and -untreated (NFA) cells as described in Fig. 1.

- ccr4Δ
- NFA
- UFA
- upf1Δ
- NFA
- UFA

G, cells grown on fatty acid-deficient medium; ○, cells exposed to 1 mM 18:2 as described under “Experimental Procedures.”
OLE1 transcript is highly stable, yielding near identical decay patterns under both basal and regulated conditions with half-lives of ~30–35 min (Fig. 6). By contrast, the OLE1 mRNA expressed under the control of the GAL1 promoter has a basal half-life of about 6.5 min, but it also showed the loss of fatty acid-regulated decay (Fig. 6). These results show that the fatty acid-regulated instability of the OLE1 transcript requires exosomal functions associated with its 3′ → 5′-mRNA decay activity. Furthermore, the striking differences between the half-lives of the transcripts produced by the native promoter and those produced by the GAL1 promoter in the ski4–1 cells suggest that there may be other mechanistic linkages involving exosome activity that contribute to OLE1 mRNA stability that are also sensitive to different modes of transcription activation.

**DISCUSSION**

This study presents the unexpected finding that the fatty acid-mediated regulation of OLE1 mRNA stability is controlled through the transcription activating factor, Mga2p. Although Mga2p and its homologue, Spt23p, lack DNA binding motifs, several lines of evidence show that they are directly involved in the activation of OLE1 transcription. For example, the expression of soluble, N-terminal fragments of either protein repair the unsaturated fatty acid auxotrophy of spt23Δ; mga2Δ cells and activate lacZ reporter genes that are under the control of the OLE1 promoter (7). Furthermore, epitope-tagged forms of Mga2p specifically associate with a complex that forms on the LORE transcription activation element of the OLE1 promoter (5).

Given that Mga2p functions in the activation of OLE1 transcription, we were surprised to find that in SPT23; mga2Δ cells, which only express the Spt23p homologue, the rate of decay of the OLE1 transcript is increased by about 2-fold and that its fatty acid-regulated instability is abolished. By contrast, Spt23p does not appear to play a role in either stabilizing the transcript or in controlling its regulated instability. For example, MGA2; spt23Δ cells do not exhibit differences in the basal or regulated half-lives of the OLE1 transcript from those in wild type cells. In addition, the reduced OLE1 basal mRNA half-life and the loss of its regulated stability in SPT23; mga2Δ cells is unaffected by the additional disruption of SPT23 in spt23Δ; mga2Δ cells. Taken together, these data demonstrate that Spt23p has little, if any, effect on the basal or regulated stability of the OLE1 transcript.

The abilities of Mga2p to contribute to the activation of OLE1 transcription and the regulation of its mRNA stability appear to be distinct and separable functions. In cells that express Mga2p, the half-lives of an OLE1 transcript expressed under the control of the unrelated GAL1 promoter are similar to those in wild type cells under basal and regulated conditions. In mga2Δ cells, however, the OLE1 transcript expressed under the GAL1 promoter shows an identical reduction in half-life and loss of regulated instability to that seen with the native OLE1 transcript. Given that Mga2p has no effect on the induction of GAL1 transcription by galactose or its rapid repression in response to glucose, these studies indicate that Mga2p exerts control over the stability of the OLE1 mRNA in the absence of its normal transcription activation functions.

These results also show that the effects of Mga2p on OLE1 mRNA stability can be separated into two distinct functions: one that results in stabilizing the transcript and another that...
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confers its regulated instability. In wild type cells grown on fatty acid-depleted medium, Mga2p is required to stabilize the transcript from a half-life of \(-5\)–\(6\) min (the case in mga2\(\Delta\) cells) to an average of 10.5 min. On exposure to unsaturated fatty acids, however, Mga2p is required to destabilize the transcript to a half-life of \(< 2\) min. Interestingly, soluble forms of Mga2p, which do not undergo proteolytic processing on the ER membrane, can only stabilize the transcript and cannot perform the destabilizing function. The inability of these soluble forms to confer fatty acid-mediated instability on OLE1 mRNA suggests that modifications introduced on the membrane-bound wild type Mga2p during its ubiquitin-mediated proteolytic processing are an essential part of the regulation mechanism.

Although it is tempting to speculate that Mga2p controls the stability of the OLE1 transcript through some type of direct mRNA binding mechanism, experiments in which we attempted to immunoprecipitate the OLE1 mRNA using N-terminal myc- and FLAG-tagged forms of Mga2p were unsuccessful under a wide range of conditions. This may be because Mga2p only binds transiently to the OLE1 ribonucleoprotein complex at some stage in its expression (e.g. transcription, messenger ribonucleoprotein (mRNP) assembly, mRNA export, or during its translation) or because Mga2p acts indirectly through some intermediate protein or proteins to control OLE1 mRNA stability.

An analysis of half-lives of the native and GAL1 induced transcripts in ski4\(\Delta\)–1 mutant cells indicates that the primary mechanism by which OLE1 mRNA stability is regulated by unsaturated fatty acids involves an activation of the degradation of the mRNA by the exosome complex. Exosomes are highly conserved multimeric particles that function both in the degradation of nuclear small RNAs and cytoplasmic mRNAs in yeast and higher eukaryotes (22–25). The yeast exosome complex consists of at least 10 protein components (25, 26) that have either a demonstrated 3’–5’-exoribonuclease activity or a high sequence homology to E. coli 3’–5’-exonucleases (27).

An analysis of cells containing the exosomal ski4\(\Delta\)–1 allele, which retains its essential nuclear RNA degradation functions but has reduced mRNA degradation activity, blocks the OLE1 fatty acid-regulated instability response in both the native and GAL1-driven mRNA decay systems. We were surprised to find that this mutation also confers a much higher level of stability on the native transcript than in wild type cells. By contrast, the same mutation reduces the half-lives of the GAL1 driven transcript to 6.5 min. It is not clear why this mutation in the RNA binding domain of the exosome should produce such a dramatic difference in the basal half-life of the OLE1 transcript expressed by the two different promoter systems. An emerging picture of exosomal function, however, indicates that in addition to its role in the cytoplasmic degradation of mRNAs, it has nuclear functions that are closely tied with transcription, mRNP assembly, and mRNA export. Recent studies, for example, have linked the nuclear exosome to the mRNA export machinery that assembles on the nascent mRNA during the early stages of transcription suggesting that it may act as a surveillance mechanism for mRNPs that are competent for nuclear export (28, 29) by degrading improperly assembled nascent mRNPs. It is conceivable that the mutation in the ski4\(\Delta\)–1 mRNA binding domain may elicit different responses of this exosomal surveillance function with respect to these two different promoter systems. This further suggests that the wild type exosome may play some role in establishing the intrinsic stability of OLE1 mRNA during transcription and mRNP assembly in addition to performing its role in the fatty acid-regulated degradation of the transcript.

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