Maintenance and Regulation of mRNA Stability of the Saccharomyces cerevisiae OLE1 Gene Requires Multiple Elements within the Transcript That Act through Translation-independent Mechanisms*

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The Saccharomyces cerevisiae OLE1 gene encodes a membrane-bound Δ-9 fatty acid desaturase, whose expression is regulated by unsaturated fatty acids through both transcriptional and mRNA stability controls. In fatty acid-free medium, the mRNA has a half-life of 10 ± 1.5 min (basal stability) that drops to 2 ± 1.5 min when cells are exposed to unsaturated fatty acids (regulated stability). A deletion analysis of elements within the transcript revealed that the sequences within the protein-coding region that encode transmembrane sequences and a part of the cytochrome b5 domain are essential for the basal stability of the transcript. Deletion of any of the three essential elements produced unstable transcripts and loss of regulated instability. By contrast, substitution of the 3′-untranslated region with that of the stable PGK1 gene did not affect the basal stability of the transcript and did not block regulated decay. Given that Ole1p is a membrane-bound protein whose activities are a major determinant of membrane fluidity, we asked whether membrane-associated translation of the protein was essential for basal and regulated stability. Insertion of stop codons within the transcript that blocked either translation of the entire protein or parts of the protein required for co-translationinsertion of Ole1p had no effect. We conclude that the basal and regulated stability of the OLE1 transcript is resistant to the nonsense-mediated decay pathway and that the essential protein-encoding elements for basal stability act cooperatively as stabilizing sequences through RNA-protein interactions via a translation-independent mechanism.

Unsaturated fatty acids are essential components of membrane lipids that determine in large part the fluid properties of membrane bilayers. In eukaryotes, unsaturated fatty acids are synthesized by fatty acid desaturases that form double bonds in fatty acyl chains by an iron-requiring, oxygen-dependent mechanism. Because of the need to stringently control the acyl composition of membrane lipids, fatty acid desaturases are highly regulated enzymes that respond to a number of stimuli, including changes in environmental temperature, nutrient fatty acids, and cellular oxygen levels. In the yeast Saccharomyces cerevisiae, unsaturated fatty acids are formed by Ole1p, a membrane-bound Δ-9 desaturase that converts long chain saturated fatty acyl-CoA substrates into monounsaturated species (1, 2). Our laboratory has shown that the OLE1 gene is regulated by a number of mechanisms, including transcriptional and mRNA stability controls (3–8).

Although most studies of gene regulation are focused on mechanisms that control the rate of transcription, mRNA degradation systems are now known to play a central role in the control of gene expression. The half-life of an mRNA species determines the number of times an individual transcript can be translated, which in turn affects the total amount of protein that can be produced by a gene at a given rate of transcription. This importance of mRNA degradation in determining the level of expression of specific genes is supported by studies that show that the decay rates of different mRNAs vary widely, and the half-lives of some transcripts have been shown to change in response to specific signals (5, 9–11).

Only a few examples of regulated mRNA decay in response to external cues have been described in yeast. Two genes from S. cerevisiae have been shown to respond to the changes in carbon source that are associated with the diauxic shift during which cells adapt to glucose depleted conditions. The SDH2 rate of mRNA turnover depends on the availability of glucose (12). When glucose is added to cells previously grown on the non-fermentable carbon source, glycerol, the half-life of the transcript drops from >60 min to ~5–7 min (10, 12, 13). By contrast, the TIFF1A transcript is stabilized in glucose-grown cells, with a half-life of 20 min that drops to 7 min when cells are transferred to glycerol-containing medium (14).

The half-life of the OLE1 transcript is also regulated when cells are exposed to unsaturated fatty acids. Yeast cells readily import long chain (C14-C16) acids and incorporate them into membrane lipids, resulting in the down-regulation of OLE1 transcription (4) and a rapid decrease in the half-life of its mRNA (5). In cells grown in fatty acid-free medium, OLE1 mRNA is a moderately stable species with a half-life of about 10 ± 1.5 min (5), whereas exposure to unsaturated fatty acids for 15 min reduces its half-life to less than 2 min.

Progress has recently been made in understanding the gen-
eral mechanisms by which the process of mRNA degradation occurs. In *Saccharomyces*, many transcripts appear to be degraded through a general pathway of mRNA decay that is initiated by deadenylation of the poly(A) tail (15–18). The subsequent displacement of poly(A)-binding proteins that stabilize the binding of translation initiation factors to the 5′ cap makes the transcript competent for decapping by the enzymes Dcp1p and Dcp2p. After removal of the 5′ cap, the mRNA is rapidly degraded by the 5′ to 3′ exonuclease, Xrn1p. Alternatively, some mRNAs can be degraded in a 3′-5′ direction by exonucleases that are associated with the exosome complex (19). The rate of the deadenylation and decapping reactions for each transcript appears to be controlled by widely divergent sequence elements in different mRNAs (17), indicating that these common functions in the general decay pathway respond to a number of different regulatory mechanisms.

Translation also appears to play an important role in governing the decay of some transcripts in the general pathway. For some mRNAs, sequences that promote efficient translation also stabilize the transcript (17, 20). For example, mutants that interfere with ribosomal loading and translation initiation promote increased rates of deadenylation (17, 18), and mutations that alter the context of the start codon can destabilize the highly stable PGK1 mRNAs.

A second translation-dependent decay pathway involves the degradation of aberrant mRNAs. In that system, the introduction of premature stop codons into protein coding sequences destabilizes some transcripts (21). The termination of translation at the nonsense codon and the assembly of a surveillance complex triggers the rapid decapping and degradation of the mRNA (22). Transcripts that are degraded by the nonsense decay pathway require the presence of a destabilizing element 3′ to the premature stop codon (23).

Elements within the 3′-untranslated region (UTR)\(^1\) of many eukaryotic mRNAs also play an important role in governing mRNA stability. The decay rate of a number of regulated transcripts are controlled by AU-rich elements (AREs) located within the 3′-UTR, and mutations in those elements have been shown to reduce the rate of poly(A) degradation and the overall rate of decay (14). The role of AREs in mRNAs appears to be somewhat variable, suggesting that they may recognize more than one type of binding protein. An ARE in the yeast MFA2 transcript, for example, destabilizes the mRNA under all tested growth conditions, whereas the ARE in the glucose-sensitive *TFI51A* transcript acts as the primary determinant of its carbon source-regulated decay (14).

An important part of understanding how mRNA stability is controlled requires identifying and characterizing the RNA elements that regulate its turnover. Unlike DNA binding proteins, trans-acting RNA-binding proteins that stabilize or destabilize transcripts rarely recognize distinct nucleotide se-sequences and instead bind to relatively long elements, which suggests that the context and secondary structure of the sequences play key roles in the process.

We previously determined that some elements involved in the OLE1 mRNA stability control mechanism are associated with the 5′-UTR of the transcript. Replacing the native 5′-UTR with sequences from the yeast GAL1 5′-UTR produces a functional OLE1 transcript with an intermediate half-life that is not regulated by unsaturated fatty acids (5). Those results suggested that the 5′-UTR of *OLE1* mRNA contains sequence elements required for fatty acid-triggered destabilization. Although these elements appear to be necessary for the generation of a normal half-life, they do not appear to be sufficient to establish the normal control of basal and regulated stability of the mRNA.

In this paper, using a series of deletion and substitution mutations, we identify multiple, widely separated elements within the protein-coding sequence of the *OLE1* transcript that are essential for its basal stability. These encode residues that are a part of the membrane-spanning regions of the protein and a part of its C-terminal cytochrome b domain. Unexpectedly, the substitution of the 3′-UTR region of the transcript with that of the highly stable *PGK1* gene does not stabilize the transcript or block its fatty acid-regulated destabilization. Given that the Δ9 desaturase is a major determinant of membrane fluid properties, we also investigated whether membrane-associated translation of the protein is involved in the mRNA stability regulation mechanism. Insertion of stop codons throughout the transcript, however, including those that block translation of the membrane-spanning regions failed to affect either basal or regulated stability of the transcript. We conclude that *OLE1* mRNA stability is regulated by a novel system that is resistant to nonsense-mediated decay and the presence of AU-rich 3′-UTR elements. We further conclude that the membrane-associated translation of the Ole1p is not an integral part of the decay process.

### EXPERIMENTAL PROCEDURES

#### Strains and Growth Conditions

*S. cerevisiae* strains used in this study are shown in Table I. *Escherichia 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 (24, 25).

Yeast cells were grown as previously described (5). The cells were grown at 30 °C in complete synthetic medium plus 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. Unsaturated fatty acids (obtained from Sigma) were added to the growth medium to a final concentration of 1 mm. Thiolutin (received as a gift from Pfizer) was added to a final concentration of 15 μg/ml.

#### DNA Isolation and Manipulations

Methods for general DNA manipulations were performed after procedures described in Ausubel et al. (24) and Maniatis et al. (25). Yeast transformations were performed using the YEASTMAKER yeast trans-
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### TABLE II

| Plasmid | Description | Source |
|---------|-------------|--------|
| pGAL-OLE2.8 | HpaI fragment of OLE1 gene containing 220 bases of 5′-UTR, entire protein-coding sequence, and 1 kb of 3′-UTR under the control of yeast GAL1 promoter | This laboratory |
| pGAL-OLE-207 | Contains 207 bp of OLE1 5′-UTR, entire protein-coding sequence, and 1 kb of 3′-UTR under the control of yeast GAL1 promoter | This laboratory |
| pGAL-OLE-BstEII1A | BstEII digestion of plasmid pGAL-OLE2.8 removes 522 bases of OLE1 protein-coding sequence that includes its TM2 | This laboratory |
| pGAL-OLE-NcoIΔ | NcoI digestion of plasmid pGAL-OLE2.8 removes 607 bases of OLE1 protein-coding sequence that includes its TM1 and TM2 and the intervening sequences | This laboratory |
| pGAL-OLE-FAHtm1 | OLE1 TM1 replaced with FAH1 TM1 in plasmid pGAL-OLE-207 | This laboratory |
| pGAL-OLE-FAHtm2 | OLE1 TM2 replaced with FAH2 TM2 in plasmid pGAL-OLE-207 | This laboratory |
| pMV088 | OLE1 protein-coding sequence deleted from the second codon to the beginning of TM1 in plasmid pGAL-OLE-207. It also has a stop codon in front of the TM1. | This laboratory |
| pMV099 | OLE1 protein-coding sequence deleted from TM2 to PacI site in plasmid pGAL-OLE-207. It also has a stop codon in the TM1. | This laboratory |
| pGAL-OLE-GFP1 | PacI site to the stop codon in OLE1 gene replaced with GFPuv protein-coding sequence in plasmid pGAL-OLE2.8 | This laboratory |
| pGAL-OLE-GFPm(2) | PacI site to the stop codon in OLE1 gene replaced with GFPmut3.1 protein-coding sequence in plasmid pGAL-OLE2.8 | This laboratory |
| pGAL-OLE-PGK3’utr | Replaced OLE1 3′-UTR with PGK1 3′-UTR in plasmid pGAL-OLE2.8 | This laboratory |
| pMV061 | ATG start codon in OLE1 gene mutated to GCG in plasmid pGAL-OLE2.8 | This laboratory |
| pMV071 | Replaced ACC with GTT at position +323 to +325 to introduce a stop codon before OLE1 TM1 in plasmid pGAL-OLE2.8 | This laboratory |
| pAM165 | Plasmid pGAL-OLE-207 was cut with PacI and Klenow-filled to introduce a stop codon through a frame-shift mutation | This laboratory |
| pE31 | Contains pGALOLE 2.8 promoter OLE1 5′-UTR, fused TM1 and TM2 sequences, cytochrome b6 domain, and 3′-UTR | This laboratory |

### Plasmid Constructions

Plasmids used in this study are described in Table II. Most of the manipulations in the OLE1 protein-coding sequence (Fig. 1) were made by PCR amplification and splicing through the splicing by overlap extension (SOE) technique (26). The constructs were verified by restriction analysis and DNA sequencing as required. Two yeast centromere-based plasmids, pGAL-OLE2.8(5) and pGAL-OLE-207, were used as parent vectors to carry out several modifications within the OLE1 gene sequences. pGAL-OLE-207 is similar to pGAL-OLE2.8 but has OLE1 gene sequences starting from base −207 of the 5′-UTR and including the entire protein-coding sequences and 1 kb of 3′-UTR fused to the GAL1 promoter through a 20-base pair linker region.

Transmembrane Loop (TM) Manipulations—Plasmids pGAL-OLE-BstEII1A and pGAL-OLE-NcoIΔ were generously provided by S. Galuska. pGAL-OLE-BstEII1A was created by complete digestion of plasmid pGAL-OLE2.8(5) with BstEII followed by isolation and religation of a 10.1-kb DNA fragment. Similarly, pGAL-OLE-NcoIΔ was constructed by complete digestion of pGAL-OLE2.8 with NcoI.

The entire FADH1 TM region of OLE1 gene was replaced by amplification of the entire FAH1 TM region using primers with an incorporated AflIII site. Plasmid p−B/HpAI was created by destroying the unique BstXI site in plasmid pCRScriptSK+. This was followed by insertion of an HpAI fragment encompassing the entire OLE1 gene cloned into the SrfI site to generate plasmid pMV055. The previous PCR product was digested with AflIII and cloned into the NotI sites of the OLE1 gene in plasmid p−B/HpAI, replacing both of its TM elements with FAH1 TMs. An OLE1 gene fragment derived from p−B/HpAI that contains all of the protein-coding sequences distal to TM1 and including 1 kb of 3′-UTR was then cloned adjacent to the FAH1 TM1 in plasmid pMV055 to generate pMV056. A SnaBI/PacI fragment from pMV056 containing the entire modified protein coding sequences was then cloned into the same sites in vector pGAL-OLE-207 to generate pMV057 (pGAL-OLE-FAHtm1).

To replace OLE1 TM2, a fragment that contains the OLE1 5′-UTR and the N-terminal part of the OLE1 protein-coding sequence (759 bp) including TM1, was amplified using primers pMV043 and pMV051 from plasmid p−B/HpAI. In a separate reaction, FAH1 TM2, part of the OLE1-coding sequence and the OLE1 3′-UTR were amplified using primers pMV052 and pMV048 from plasmid pMV055. The two PCR products were fused by the SOE technique followed by digestion with restriction enzymes XbaI/HindIII and insertion into the same sites in plasmid pGAL-OLE-207 to create pGAL-OLE-FAHtm2.

To replace both OLE1 TMs, a fragment that contains the OLE1 5′-UTR, the N-terminal part of OLE1 protein-coding sequence linked to FAH1 TM1, and part of the OLE1 sequence between the TM loops was amplified using primers pMV043 and pMV051 from plasmid pMV056. In a separate reaction, a fragment containing the remaining elements of the OLE1 intermembrane loop sequences, FAH1 TM2, the C-terminal part of the OLE1 protein-coding sequence, and the OLE1 3′-UTR was amplified using primers pMV052 and pMV048 from plasmid pMV055. The two fragments were then fused by the SOE technique, digested with restriction enzymes XbaI/HindIII, and inserted into the same sites in plasmid pGAL-OLE-207 to create pGAL-OLE-FAHtm1&2.

Deletion of Sequences from the Start Codon to the Beginning of Transmembrane Loop 4 (Plasmid pMV088) The OLE1 5′-UTR (220 bp) was amplified by PCR using primers pMV043 and pMV132 from plasmid pGAL-OLE2.8. In a second reaction the OLE1-coding sequences beginning of the OLE1-coding sequence were deleted past the start of TM regions (+301 bp) and OLE1 3′-UTR (1 kb) was amplified using primers pMV132 and pMV048 from plasmid pMV071. Both PCR products were spliced by SOE and cloned into SrfI site in plasmid pGAL-OLE-207.
Deletion of the Protein Coding Sequence between the Transmembrane Loops of OLE1 (pMV089)—A 716-bp fragment that contains the OLE1 5'-UTR (220 bp) and protein-coding sequence to the end of OLE1 TM1 was amplified by PCR using the primers prMV043 and prMV134 from plasmid pMV071. In a second reaction, the OLE1-coding sequence that includes TM2 and 3'-UTR (1 kb) was amplified using primers prMV135 and prMV048 from plasmid pGAL-OLE2.8. The PCR products were spliced by SOE, and the resultant product was cloned into the SrfI site in pCRScriptSK+ plasmid to make pMV086. The XbaI/HindIII fragment (2453 bp) of pMV086 was then cloned into the same sites on pGAL-OLE-GFP1.

Deletion of Protein-coding Sequence between Transmembrane Loop 2 and 3UTr Site—Primers prMV043 and prMV138 were used to amplify 220 bp of OLE1 5'-UTR and 918 bp of OLE1 protein-coding sequence until the end of TM2 using plasmid pMV071 as a template. In another PCR reaction, primers prMV139 and prMV048 were used to amplify 195 bp of OLE1 protein-coding sequences starting from Plac site to the stop codon and 1 kb of 3'-UTR using plasmid pB-Hpal as template. The PCR products were spliced by SOE, and cloned into the SrfI site in pCRScriptSK+ plasmid to make pMV087. The XbaI/HindIII fragment of pMV087 (2306 bp) was then cloned into the same sites in pGAL-OLE2.8 plasmid vector to generate pMV090.

Substitution of OLE1-coding Sequence from PacI Site in the Cytochrome b₅-like Region to the Stop Codon with GFP Protein-coding Sequences—The GFP protein-coding sequence from plasmid GFPmut3.1 was amplified by PCR using primers prMV072 and prMV074. In a separate reaction the OLE1-coding sequence beyond the Plac site in the cytochrome b₅-like region to the stop codon and OLE1 3'-UTR was amplified with primers prMV075 and prMV048 from plasmid pB-Hpal. The PCR products were spliced by SOE and cloned into the SrfI site in pCRScriptSK+ plasmid to make PCR-GFPm (2). A Plac/HindIII fragment from plasmid PCR-GFPm (2) was ligated into same sites in plasmid pGAL-OLE2.8 to generate plasmid pGAL-OLE-GFPm (2).

The OLE1 protein-coding sequence from Plac site to the stop codon was amplified using primers from the GFP protein-coding sequence from plasmid pGFPuv (Clontech). A 716-bp fragment containing the GFP-coding sequence was amplified from pGFPuv with primers prMV068 and prMV069. In a separate reaction the OLE1 protein-coding sequences from the Plac site to the stop codon and OLE1 3'-UTR was amplified using primers prMV070 and prMV048 from plasmid pB-Hpal. The two PCR products were spliced by SOE and cloned into the SrfI site in plasmid pCRScriptSK+ plasmid to generate PCR-GFP1. Plac/HindIII digestion of PCR-GFP1 released a 1716-bp fragment that was then cloned into same sites on pGAL-OLE2.8 to make plasmid pGAL-OLE-GFP1.

Substitution of TM1, TM2, and Cytochrome b₅ Essential Elements within the OLE1 5' - and 3' -UTR Regions—The HpaI fragment of OLE1 containing the entire mRNA-encoding sequence was digested with Sall/PacI to remove elements of the OLE1 protein-coding sequence extending from the N-terminal Sall site to the Plac site within the cytochrome b₅ domain. PCR-amplified fragments encoding the TM1 and TM2 regions were fused by the introduction of a Sall restriction site in the forward PCR primer and a PacI site in the reverse primer to construct vectors pGAL-OLE1-TM1-cyto₅, pGAL-OLE1-TM2-cyto₅, and vector pE31, which contain all three essential elements of the protein-coding sequence.

Replacing the OLE1 3'-UTR with the PGK1 3'-UTR (Plasmid pGAL-OLE-PGK3 utr)—The OLE1 protein-coding sequence extending from +1 to 1353 bp was amplified with primers prMV060/prMV069 from plasmid pGAL-OLE2.8 and the PGK1 3'-UTR (1 kb after the stop codon) was amplified by PCR with primers prMV034/prMV035 from genomic DNA (W3031A). Each of the PCR fragments was cloned separately into the SrfI site in pCRScriptSK+ to generate plasmids pMV048 (containing the OLE1-coding sequence) and pMV049 (containing PGK1 3'-UTR). A 1.3-kb fragment of pMV049 released by SrfI digestion was inserted into Aatt1/EcoRV sites in plasmid vector pMV048 behind the OLE1-coding sequence. The resulting plasmid pMV050 was then digested with XhoI and Klenow-filled to blunt end followed by PacI cut to release 2533-kb fragment. This fragment was inserted into HindIII (Klenow-filled)/PacI sites in pGAL-OLE2.8 to generate plasmid pGAL-OLE-PGK3 utr.

Mutation to Introduce GCC in Place of Translational Start Codon ATG (Plasmid pMV061) in OLE1 Gene—OLE1 start codon was mutated through PCR technique. Primers prMV059 and prMV060 are complementary and a three-base pair change compared with the original sequence was incorporated to facilitate the translation of the ATG start codon to GCC. OLE1 5'-UTR (937 bp) was amplified using primers AGM116 and prMV060. In a separate reaction the OLE1-coding sequence and 3'-UTR was amplified using primers prMV059 and prMV062. The two PCR products were spliced by SOE followed by SnaBl/PacI digestion. This fragment was then cloned into the same sites in pGAL-OLE2.8 to make plasmid pMV061.

Introduction of a Stop Codon through a Frameshift Mutation in the Cytochrome b₅-like Region in OLE1 Protein-coding Sequence—Plasmid pGAL-OLE2.8 was cut with PacI, filled-in by Klenow polymerase, and religated to produce pAM165. The resulting plasmid contains a frameshift mutation that introduces a stop codon between residues +1353 to +1355 within the cytochrome b₅ domain of Ole1p.

mRNA Decay Measurements

mRNA decay rates were essentially determined as previously described (5). Transcription was arrested either by using transcriptional inhibitor thiolutin (15 µg/ml) or through glucose repression of the yeast GAL1 promoter. RNA isolations and quantitative Northern blot analysis of cells after transcription arrest were performed as previously described (5).

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 Yepl532-OLE4.5. The OLE1 FAH1 dimeric mRNAs were detected by probing against either one or both of the substituted FAH1 transmembrane loops. The DNA templates for FAH1 probe preparation were prepared by PCR. Primers used to amplify TM1 (506 bp) are prMV056 and prMV057, and primers used to amplify TM2 (501 bp) are prMV041 and prMV058. Probes for GFP detection were made from a template amplified from plasmid pGFPmut3.1 (Clontech). The primers used are prMV072 and prMV074. The DNA template for GFP1 probe was amplified using the primers prMV016 and prMV017. For all experiments the internal control was provided by a 1.0-kb HindIII-KpnI fragment of the phosphoglycerate kinase (PGK1) gene isolated from plasmid pIP1P0GK (27) unless specifically stated. All DNA fragments were separated on agarose gel and electrophoresed in 1x TAE buffer and purified by Gene Clean II (Bio101). The DNA fragments were labeled to high specific activity with [α-32P]dATP (PerkinElmer Life Sciences) by the random primer extension method using 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.

RESULTS

OLE1 mRNA Degradation under Basal and Regulated Conditions Involves Decapping by Dcp1p and Xrn1p-dependent Exonuclease Digestion—Recent studies show that many yeast mRNAs are degraded by a general deadenylation-dependent pathway that involves removal of the 5′ cap by the Dcp1p-Dcp2p complex and subsequent degradation by the 5′-3′ exonuclease, Xrn1p (17, 18, 28). An important question was whether the basal and regulated decay of the native OLE1 transcript occurred through that pathway or through an alternative mechanism. Fig. 2 shows the effects of null mutations in the XRN1 and DCP1 genes on OLE1 mRNA decay. Panel A shows that in wild type cells the OLE1 transcript decays with a half-life of 10 min in fatty acid-free medium and 2.5 min on medium containing 1 mM linoleic acid (18:2) Sigma. In xrn1Δ cells (panel B) the lifetime of the transcript is dramatically increased to 45 and 23 min under the same conditions, indicating that fatty acids continue to exert some regulation even
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Deletion and Substitution Analysis of OLE1 mRNA Reveals Sequences in the Protein-coding Region That Are Required for Basal and Regulated Stability—Previously we reported that the 5′-UTR of OLE1 mRNA is required for its basal and unsaturated fatty acid-mediated stability (5). This element, however, is insufficient to confer the wild type pattern of basal and regulated stability on heterologous mRNAs. To identify other essential elements that reside in the protein-coding or 3′-UTR regions of the mRNA we performed a deletion and substitution analysis of the entire transcript. The relationship of the structural domains of Ole1p to features within the protein-coding region of the OLE1 transcript that are relevant to those experiments are shown in Fig. 1. Ole1p is a bifunctional protein that consists of an N-terminal fatty acid desaturase domain fused to a C-terminal cytochrome b5 domain. The protein is anchored to the membrane through two hydrophobic regions within the desaturase domain, and each hydrophobic element appears to traverse the membrane as a loop, so that the active sites of the two functional domains are located on the cytosolic side of the membrane.

Two initial test vectors that were constructed for these experiments contained deletions in sequences that encode the TM loop elements. TM loop 1 is positioned between amino acids 116 and 159, and TM loop 2 is positioned between amino acids 257 and 305 (Fig. 1) (2).

Plasmid pGAL-OLE-BstEIIΔ contains a 522-bp internal deletion that encodes amino acids 159 to +333. The polypeptide encoded by that plasmid retained loop region 1, but the deletion removed all of the sequences positioned between the TM loops and loop 2 (Figs. 1 and 3b). Plasmid pGAL-OLE-NcoIΔ contains a 607-bp internal deletion that encodes residues from +101 to +303. The polypeptide that was encoded by that plasmid lacked both TM domains of the desaturase (Figs. 1 and 3c). Although these deletions did not alter the reading frames, neither plasmid would support growth of an ole1Δ gene-disrupted strain on fatty acid-free medium, indicating that both encoded a non-functional protein (data not shown).

Both vectors produced the mutant OLE1 transcripts under control of the GAL1 promoter, which is inducible by galactose and can be rapidly arrested by the addition of glucose. The mRNA decay measurements in wild type cells containing those plasmids revealed that they produced unstable transcripts, with half-lives of ~3 min in the presence and absence of unsaturated fatty acids (Fig. 4) compared with the native transcripts (inhibited with the RNA polymerase inhibitor, thiolutin), which exhibits the normal basal half-life of 10 min and a regulated half-life of 2.5 min.

Essential Elements for Basal and Regulated Decay within the OLE1 Protein-coding Region Include the Transmembrane-encoding Elements and Sequences Associated with the C-terminal Part of the Cytochrome b5 Domain—The results from the above deletion analysis suggested that specific elements within the transmembrane-encoding sequences might play a role in stabilizing the transcript and in the regulation of its stability. To test the possibility that the membrane-spanning sequences were essential for stability regulation, the OLE1 TM loop regions were replaced with non-homologous sequences (~30% nucleotide sequence identity and ~21% peptide sequence identity) that encode membrane-spanning regions from the Saccharomyces FAH1 gene. Fah1p is a membrane-bound fatty acid biosynthetic enzyme that hydroxylates 26-carbon fatty acids (29). It is a bifunctional, cytochrome b5 fusion protein that has similar structural features to Ole1p. In Fah1p the fused cytochrome b5 domain is located at the N terminus of the protein. The C-terminal catalytic domain, however, contains two hydrophobic TM loop regions that are topologically analogous to Ole1p. TM loop sequences 1 and 2 of FAH1 encode 30 and 37 amino acids, whereas the OLE1 TM loop regions encode 45 and 49 amino acids.
Three chimeric plasmids were made by replacing one or both of the OLE1 transmembrane loops with the relevant FAH1 sequences (Fig. 5). Plasmids pGAL-OLE-FAHtm1 and pGAL-OLE-FAHtm2 contained replacements of the single transmembrane loop regions; plasmid pGAL-OLE-FAHtm1&2 contained substitutions in both regions.

The half-lives of the mRNAs produced from the three constructs expressed in fah1/H9004 strains were determined using RNA probes complementary to the inserted FAH1 sequences (Fig. 5). All three chimeric genes produced unstable mRNAs with half-lives of 3–4 min in cells exposed to either fatty acid free or 18:2-supplemented growth medium. This strong correlation of the decay pattern with those from the transcripts generated from the TM loop deletion constructs further supports that sequences in the OLE1 transmembrane loop regions are essential elements for the basal and regulated stability of the transcript.

A stability-determining element was also uncovered within the cytochrome b5 domain of the OLE1 protein-coding sequence when sequences between the PacI restriction site to the C terminus were replaced with GFP protein-coding sequences. Transcripts generated from two plasmid constructs, pGAL-OLE-GFP1 and pGAL-OLE-GFPm (2), showed that both mRNAs are very unstable and decay with a half-life of about 3 min in the presence and absence of fatty acids (Fig. 6).

By contrast deletion analysis revealed that the sequences that separate the stabilizing TM1, TM2, and cytochrome b5 elements do not play a direct role in either basal or regulated mRNA stability. Plasmids pMV088, pMV089, and pMV090, respectively, contain deletions of the sequences from the start codon to TM1, the sequences between the transmembrane loops, and a sequence from TM2 to a PacI site within the cytochrome b5-like domain (Fig. 7). The half-life of transcripts produced from all three plasmids is 13–14 min in the absence of fatty acids and 4 min in the presence of 1 mM 18:2 (Fig. 7).

The Context and Spacing of the Essential Stabilizing Sequences Play a Role in Determining Basal and Regulated Stability—The essential stabilizing TM1, TM2, and cytochrome b5 elements were tested by placing them between the OLE1 5′- and 3′-UTR regions to determine whether they were sufficient to establish the wild type pattern of mRNA decay. A series of vectors containing individual TM1, TM2 sequences, and the TM1 and TM2 elements fused together generated highly unstable and unregulated transcripts with half-lives of <3 min under basal and regulated conditions (data not shown). Then, to determine whether linkage of all of the essential elements was sufficient to exert the wild type pattern of control, we constructed vectors that contain all three stabilizing sequences placed between the 5′- and 3′-UTR regions (Fig. 8). Vector pE31 contains the fused TM1, TM2, and cytochrome b5 regions.

Fig. 4. Basal and regulated decay kinetics of OLE1 transcripts containing wild type and deleted transmembrane loop sequences. Phosphor images of Northern blots of total RNA isolated from wild type strain DTY10 containing plasmids pGAL-OLE-BstEIΔ (a) and pGAL-OLE-NcoIΔ (b). Transcription was induced by adding 2% galactose to the growth medium and arrested by adding 2% glucose and 15 μg/ml thiolutin. The blots were probed with OLE1 gene sequences, stripped, and reprobed with PGK1 sequences used as an internal control. Two OLE1 transcripts are detected in each experiment. The longer form (A) is the native mRNA (inhibited by thiolutin), and the shorter form (B) contains deletions in the transmembrane loops regions (inhibited by glucose). c and d, plots of decay rates of deleted transcripts shown in panels a and b in the absence (c, t1/2 = 2.5 min) and the presence of (d, t1/2 = 2.5 min) of 1 mM 18:2. Data was normalized to the corresponding steady state levels of PGK1 and plotted as the log of percentage mRNA remaining versus time after transcription arrest. Lines through data points indicate 2nd order regression curves, r² ≥ 0.9.
flanked by the OLE1 5'- and 3'-UTR regions. Analysis of the chimeric transcript, however, revealed that they have a half-life of <3 min under both basal and regulated conditions. We also constructed and tested vectors in which the sequences that separate those elements were replaced by corresponding FAH1 gene sequences. Those chimeric transcripts also showed similar instabilities under all conditions tested (data not shown). Taken together, these data indicate that the stabilizing elements within the protein-coding regions are necessary, but not sufficient, to determine the wild type levels of basal and regulated stability. Furthermore, although the individual regions that flank the TM and cytochrome b5 elements are not essential for the wild type patterns of mRNA stability, the context and spacing of those intervening sequences appear to have some role in determining the wild type pattern of OLE1 mRNA decay, possibly by providing essential spacing between a minimum number of the essential elements or through secondary structure contributions to the transcript.

FIG. 5. Decay of chimeric OLE1 mRNAs containing FAH1 TM elements. a–d, schematic diagrams illustrating substituted sequences in each plasmid and corresponding decay patterns. All the constructs were under the control of GAL1 promoter elements (gray box) that include 57 bases of the GAL1 5'-UTR. The straight line represents OLE1 gene sequences, and the dashed line represents the vector sequences. The GAL1 promoter sequences are fused through a 20-base pair linker region to the OLE1 sequences at base 207. a, parent plasmid pGAL-OLE-207 containing wild type OLE1 mRNA-encoding sequences. b, plasmid pGAL-OLE-FAHtm1, 177 bp of the OLE1-coding sequence (from base +302 to base +479) that encodes TM1 was replaced with 250 bp of FAH1 TM1 sequences (from base +575 to base +825). c, in plasmid pGAL-OLE-FAHtm2, 144 bp (from base +766 to base +910) of OLE1 TM2-coding sequence was replaced with 126 bp of FAH1 TM2 coding sequence (from base +853 to base +979). d, in plasmid pGAL-OLE-FAHtm1&2, both the transmembrane domains were replaced with FAH1 TM sequences used in b and c. Transcription of the chimeric mRNAs was induced with 2% galactose and arrested with 2% glucose as described in Fig. 4. The chimeric RNAs were hybridized to probes made from the same FAH1 TM sequences that were substituted in each plasmid. There are two transcripts detected by the FAH1 probes. The longer one is the OLE1::FAH1 chimeric transcript, and the shorter one is a part of the FAH1 transcript fragment that is disrupted with the LEU2 gene.

FIG. 6. Decay of OLE1 transcripts with C-terminal protein-coding sequences substituted with GFP sequences. Phosphor images of Northern blots of total RNA isolated from pGAL-OLE-GFP1/DTY10A (a) and pGAL-OLE-GFPm (2)/DTY10A (b) strains. Total mRNA from cultures induced by galactose and repressed by glucose were analyzed as described in Figs. 3 and 5. The blots were probed with GFP gene sequences obtained from plasmid pGFPmut3.1 (Clontech) and PGK1 sequences, used as an internal control.
a manner that is dependent on the fatty acid composition of the lipid bilayer.

To test the hypothesis that translation through the transmembrane loop sequences is essential for basal and regulated mRNA decay, vectors were constructed that would produce full-length OLE1 mRNAs with stop codons inserted at different positions within the protein-coding sequence. Vector pMV071 contains a stop codon immediately before the TM loop 1-encoding region by replacing bases ACC with GTT. The resulting mRNA has unaltered sequences within the transmembrane loop regions and encodes a 107-residue soluble N-terminal polypeptide. Analysis of the decay kinetics of the mutant transcript in wild type cells showed that it exhibits half-lives of 10.5 and 3.5 min under basal and regulated conditions, which are identical to those of the native mRNA species (Fig. 9b). These results indicate that the mRNA sequences within the transmembrane loop regions and not the protein encoded by those elements are essential for basal and fatty acid-regulated stability.

Plasmids containing other modifications of the protein-coding region showed that the context of the start codon or translation of the cytochrome b5 region was not required for basal or fatty acid-mediated regulation of mRNA stability. A plasmid in which the /H11001 ATG start codon was replaced with a GCG sequence (plasmid pMV061) and one in which a stop codon was introduced into the cytochrome b5 domain (plasmid pAM165) both produced full-length, but non-functional transcripts that could not rescue the /H9004 strain (Fig. 9a). A kinetic analysis of both transcripts showed that they decay with the same basal and regulated half-lives as the wild type mRNA. (Fig. 9, c and d). Thus, it appears that translation of the entire OLE1 polypeptide is not essential for basal or regulated stability of the transcript.

The 3'-UTR Region Is Not Essential for Basal or Regulated Stability of the OLE1 Transcript—Sequences in the 3'-UTR regions have previously been shown to be involved in mRNA stability regulation of many yeast and mammalian genes (15, 20, 31, 32). An analysis of expressed sequence tag data indicates that the OLE1 3'-UTR region includes an essential “efficiency element” (UAUAUA) 252 bases downstream of the stop codon. Within that region are multiple ARE sequences. To determine whether these and other AREs are essential regu-

![Image](https://example.com/image.png)
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The short basal half-life of the OLE1 transcript and its fatty acid-regulated instability indicates that mRNA degradation is an important component of OLE1 gene expression (5). Although this system has common features with the terminal steps of the general decay pathway, its basal and regulated stability appear to be controlled by a system that differs significantly from previously described systems. The dramatic increase in the stability of the OLE1 transcript in dep13Δ and xrn1Δ mutants (5) shows that under basal and regulated conditions degradation involves the removal of the 5′ cap, which is then followed by 5′-3′ digestion by the Xrn1p exonuclease. Although the >10-fold increase in the OLE1 regulated half-life in xrn1Δ cells indicates that Xrn1p digestion is the primary mode of OLE1 degradation under normal growth conditions, it appears that other nucleases also contribute to regulated OLE1 decay. This is supported by the observation that the OLE1 transcript decays about twice as fast in 18:2-treated xrn1Δ cells as it does in xrn1Δ cells growing in fatty acid-free medium. One candidate for this alternative degradation pathway might be the recently described yeast exosome, a complex of 3′-5′ exoribonucleases (19, 33–35).

To understand how the half-life of OLE1 mRNA is determined, we attempted to identify the cis elements that control its stability. We previously reported that the OLE1 5′-UTR is only partially attenuated by the substituted 3′-UTR, resulting in a 6-min half-life when cells are exposed to 18:2.

FIG. 9. Test of plasmids carrying mutant OLE1 genes with pre-mature stop codons. a, plasmids pMV071, pMV061, and pAM165 were transformed into ole1 HpaI::LEU2, DTY-10A strain. The transformants were rescued on leucine, uracil drop-out galactose plates with and without 1 mM 18:2 to test for desaturase function. b–d, mRNA decay patterns of mutant transcripts pMV071 (stop codon before TM1), pMV061 (mutant start codon), and pMV165 (stop codon within cytochrome b3 region).

FIG. 10. Decay of OLE1 transcript containing a 3′-UTR from the PGK1 gene. Total mRNA from cultures induced by galactose and repressed by glucose was analyzed as described in Figs. 4 and 5.

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DISCUSSION

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sense-mediated decay. That element is the apparent binding site of the RNA-binding protein Pub1p (22). Neither the PGK1 nor the GCN4 element has significant homologies to the stabilizing sequences in the OLE1 protein-coding region, which suggests that the essential OLE1 sequences represent a different class of mRNA elements.

Previous studies show that for many mRNAs there is a strong linkage between translation and mRNA turnover (11, 17, 39). For example, translation of ~55% of the PGK1 mRNA is required for its stabilizing elements to be functional (37). Other studies of the PGK1 mRNA show that a critical step in the triggering degradation involves competition between the cap binding complex, which promotes translation initiation, and the decapping enzyme Dcp1p (17, 18). The rate of decapping and degradation of that transcript is highly sensitive to its translation efficiency (18, 20), and changes in the sequence context of the PGK1 start codon that reduce its rate of translation initiation dramatically destabilize the mRNA.

The studies presented here, however, indicate that the half-life of the OLE1 mRNA is remarkably insensitive to its translation. It is resistant to changes in the context of the native start codon to nonsense-mediated decay and is not destabilized by the insertion of stop codons placed throughout the protein-coding region. These results further demonstrate that the OLE1 mRNA stability determinants have novel features that differ from those identified with other yeast mRNAs.

Another type of translation-dependent decay mechanism involves the autoregulation of mammalian β-tubulin mRNA stability. That system involves a mechanism in which excess free β-tubulin subunits bind to the N-terminal residues of the nascent polypeptide as it emerges from the ribosome, destabilizing the mRNA (30). Although the exact molecular mechanism that triggers the rapid decay of the transcript is not known, an important feature of the regulation system is that the N terminus of the partially translated protein acts as a sensor that can trigger the degradation of the ribosome-bound mRNA.

The identification of essential stability elements in the OLE1 transmembrane loop regions suggested that the nascent Ole1p polypeptide sequences might play a similar role in regulating mRNA stability. During translation of its membrane-spanning regions, OLE1 polypeptides must remain tightly associated with the endoplasmic reticulum membrane surface, and the association of the nascent Ole1p polypeptides with the lipid bilayer could offer a novel mechanism for controlling its stability through some type of membrane fluidity regulation mechanism.

The lack of response to stop codons placed throughout the OLE1 transcript, however, indicates that its degradation is not dependent on translation of the native Ole1p polypeptide and that translation of the essential stability elements is not essential for basal and regulated decay. These findings argue that the RNA sequences in the essential regulatory regions rather than the translated protein is the molecular basis of control over the half-life of the OLE1 transcript. These apparently serve as recognition sites for RNA-binding proteins that stabilize the transcript in the absence of fatty acids and promote rapid decapping and Xm1p exonuclease digestion when cells are exposed to fatty acids.

A most surprising finding is that the OLE1 3′-UTR has little effect on the basal or regulated stability of the transcript. Most studies of eukaryotic mRNA stability indicate that elements in the 3′-UTR of transcripts are primary determinants of mRNA half-lives. A number of transcripts in yeast and mammalian cells have been shown to be tightly regulated through AU-rich elements that are present in their 3′-UTRs, and these both yeast and mammalian AU-rich elements promote deadenyla-

tion-dependent decapping in yeast systems (14). The OLE1 3′-UTR contains a number of AU-rich sequence elements consisting of five pentamer (AUUUAU) and two heptamer (UAUUUAU) consensus sequences within the UTR region. Thus, it was surprising to find that replacement of the OLE1 3′-UTR with the 3′-UTR of the highly stable PGK1 gene did not alter the basal stability of the transcript nor did it abolish the fatty acid-regulated decay.

This study describes the identification of multiple cis elements that are required for the unsaturated fatty acid-mediated regulation of OLE1 mRNA stability and demonstrates that this regulation system has novel features compared with those shown for other yeast mRNAs. Besides OLE1, only the yeast SDH2 (12) and TIF51A (14) genes have been shown to exhibit instability that is affected by changes in nutritional or physiological conditions. The Ole1p fatty acid desaturase plays a central role in the formation of membrane lipids and the control of the physical properties of membrane lipid bilayers, and it is regulated by a complex system of controls at both the levels of transcription and mRNA stability. Given the importance of eukaryotic fatty acid desaturases to the maintenance and assembly of cellular membranes as well as in the production of fatty acid derived signaling molecules, it is not surprising that multiple systems have evolved for the control of these enzyme systems. The identification and characterization of proteins that bind to the OLE1 elements and the determination of the role that their structure and context play in the regulation system should provide important insights into how this system contributes to the control of eukaryotic lipid metabolism.

Acknowledgments—We thank Dr. Fredrick L. Naidu and Donnie W. Owens, Pfizer, Inc. for the gift of thiolutin for use in this project. We also extend our thanks to Dr. Andrew Mitchell for his thoughtful comments.

REFERENCES

1. Stukey, J. E., McDonough, V. M., and Martin, C. E. (1989) J. Biol. Chem. 264, 16537–16544
2. Stukey, J. E., McDonough, V. M., and Martin, C. E. (1990) J. Biol. Chem. 265, 20144–20149
3. Bosio, M. A., and Martin, C. E. (1989) J. Bacteriol. 171, 6409–6413
4. Choi, J. Y., Stukey, J., Hwang, S. Y., and Peltz, S. W. (1995) J. Biol. Chem. 271, 3581–3589
5. Gonzalez, C. I., and Martin, C. E. (1996) J. Biol. Chem. 271, 25801–25809
6. Jiang, Y., Vasconcelles, M. J., Wretzel, S., Light, A., Martin, C. E., and Goldberg, M. A. (2001) Mol. Cell. Biol. 21, 6161–6169
7. Vasconcelles, M. J., Jiang, Y., McDavid, K., Ghobry, L., Wretzel, S., Porter, D. L., Martin, C. E., and Goldberg, M. A. (2001) J. Biol. Chem. 276, 14374–14384
8. Chellappa, R., Kandassamy, P., Oh, C.-S., Jiang, Y., Vemula, M., and Martin, C. E. (2001) J. Biol. Chem. 276, 45548–45556
9. Beelman, C. A., and Parker, R. (1995) Cell 81, 179–183
10. Lombardo, A., Cereghino, G. P., and Scheffner, I. E. (1992) Mol. Cell. Biol. 12, 2941–2948
11. Mitchell, P., and Tollervey, D. (2001)Curr. Opin. Cell Biol. 13, 320–325
12. Prieto, S., de la Cruz, B. J., and Scheffner, I. E. (2000) J. Biol. Chem. 14155–14166
13. Cereghino, G. P., Atencio, D. P., Saghibini, M., Beiner, J., and Scheffner, I. E. (1995) Mol. Cell. Biol. 6, 1125–1134
14. Vasconcelles, M. J., and Peltz, S. W. (2003) Mol. Cell.7, 1191–1200
15. Decker, C. J., and Parker, R. (1993) Genes Dev. 7, 1632–1643
16. Muhlrad, D., Decker, C. J., and Parker, R. (1995) Mol. Cell. Biol. 15, 2145–2156
17. Schwartz, D. C., and Parker, R. (1999) Mol. Cell. Biol. 19, 5247–5256
18. Schwartz, D. C., and Parker, R. (2000) Mol. Cell. Biol. 20, 7893–7942
19. Jacobs, J. S., Anderson, A. R., and Parker, R. R. (1998) EMBO J. 17, 1457–1506
20. LaGrandeur, T., and Parker, R. (1999) RNA (N.Y.) 5, 420–433
21. Hagan, K. W., Ruiz-Echevarria, M. J., Quan, Y., and Peltz, S. W. (1995) Mol. Cell. Biol. 15, 809–823
22. Ruiz-Echevarria, M. J., and Peltz, S. W. (2000) Cell 101, 741–751
23. Ruiz-Echevarria, M. J., Gonzalez, C. I., and Peltz, S. W. (1998) EMBO J. 17, 575–589
24. Arvieux, F. M., Brent, R., Kingon, E. R., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York
25. Manuzio, T., Fritsch, E. F., and Sambrook, J. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
26. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1988)
Regulated mRNA Stability

27. He, F., Peltz, S. W., Donahue, J. L., Rosbash, M., and Jacobson, A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7034–7038
28. Steiger, M., Carr-Schmid, A., Schwartz, D. C., Kiledjian, M., and Parker, R. (2003) RNA (N. Y.) 9, 231–238
29. Mitchell, A. G., and Martin, C. E. (1997) J. Biol. Chem. 272, 28281–28288
30. Yen, T. J., Machlin, P. S., and Cleveland, D. W. (1988) Nature 334, 580–585
31. Heaton, B., Decker, C., Muhlrad, D., Donahue, J., Jacobson, A., and Parker, R. (1992) Nucleic Acids Res. 20, 5365–5373
32. Muhlrad, D., and Parker, R. (1992) Genes Dev. 6, 2100–2111
33. Allmang, C., Petfalski, E., Podtelejnikov, A., Mann, M., Tollervey, D., and Mitchell, P. (1999) Genes Dev. 13, 2148–2158
34. Bousquet-Antonelli, C., Presutti, C., and Tollervey, D. (2000) Cell 102, 765–775
35. van Hoof, A., and Parker, R. (1999) Cell 99, 347–350
36. Caponigro, G., Muhlrad, D., and Parker, R. (1993) Mol. Cell. Biol. 13, 5141–5148
37. Ruiz-Echevarria, M. J., Munshi, R., Tomback, J., Kinzy, T. G., and Peltz, S. W. (2001) J. Biol. Chem. 276, 30995–31003
38. Mitchell, P., and Tollervey, D. (2000) Curr. Opin. Genet. Dev. 10, 193–198
Maintenance and Regulation of mRNA Stability of the \textit{Saccharomyces cerevisiae} \textit{OLE1} Gene Requires Multiple Elements within the Transcript That Act through Translation-independent Mechanisms

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\textit{J. Biol. Chem.} 2003, 278:45269-45279.
doi: 10.1074/jbc.M308812200 \textit{originally published online} August 28, 2003

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