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Shuyun Dong
University of Massachusetts Medical School

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Degradation of YRA1 Pre-mRNA in the Cytoplasm Requires Translational Repression, Multiple Modular Intronic Elements, Edc3p, and Mex67p

Shuyun Dong, Allan Jacobson, Feng He*

Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America

Abstract

Intron-containing pre-mRNAs are normally retained and processed in the nucleus but are sometimes exported to the cytoplasm and degraded by the nonsense-mediated mRNA decay (NMD) pathway as a consequence of their inclusion of intronic in-frame termination codons. When shuttled to the cytoplasm by autoregulated nuclear export, the intron-containing yeast YRA1 pre-mRNA evades NMD and is targeted by a cytoplasmic decay pathway mediated by the decapping activator Edc3p. Here, we have elucidated this transcript-specific decay mechanism, showing that Edc3p-mediated YRA1 pre-mRNA degradation occurs independently of translation and is controlled through five structurally distinct but functionally interdependent modular elements in the YRA1 intron. Two of these elements target the pre-mRNA as an Edc3p substrate and the other three mediate transcript-specific translational repression. Translational repression of YRA1 pre-mRNA also requires the heterodimeric Mex67p/Mtr2p general mRNA export receptor, but not Edc3p, and serves to enhance Edc3p substrate specificity by inhibiting the susceptibility of this pre-mRNA to NMD. Collectively, our data indicate that YRA1 pre-mRNA degradation is a highly regulated process that proceeds through translational repression, substrate recognition by Edc3p, recruitment of the Dcp1p/Dcp2p decapping enzyme, and activation of decapping.

Introduction

mRNA degradation controls the level of gene expression and ensures transcript quality control. In the yeast Saccharomyces cerevisiae, most wild-type mRNAs are degraded by the general 5′ to 3′ or 3′ to 5′ decay pathways [1]. Functionally impaired mRNAs are targeted for degradation by several translation-dependent mRNA surveillance mechanisms, including nonsense-mediated mRNA decay (NMD) for mRNAs containing premature termination codons [2], non-stop decay (NSD) for mRNAs lacking translation termination codons [3,4], and no-go decay (NGD) for mRNAs stalled in translational elongation [5]. Transcript-specific decay pathways have also been identified in several experimental systems [6,7]. In each of these pathways, degradation of a transcript is regulated by specific cis-acting elements and their respective trans-regulatory RNA-binding factors. For example, adenine/uridine-rich elements (AREs) have been found in the 3′-untranslated regions (3′-UTRs) of diverse eukaryotic mRNAs [8], and these elements by themselves, or through their interacting proteins, can accelerate transcript-specific decay by recruitment of the PARN and Ccr4p deadenylases [9,10], the exosome [11–13], or the Dcp1p/Dcp2p decapping enzyme [9]. Our recent experiments, and those of Badis et al., have identified a yeast cytoplasmic, transcript-specific decay pathway, mediated by the decapping activator Edc3p, that principally targets only two transcripts, RPS28B mRNA and intron-containing YRA1 pre-mRNA [14,15].

Intron-containing pre-mRNAs are normally retained and processed in the nucleus [16,17] but are sometimes exported to the cytoplasm where their inclusion of intronic in-frame termination codons targets these transcripts for degradation by the NMD pathway [18,19]. However, the intron-containing YRA1 pre-mRNA evades NMD and is degraded by the Edc3p-mediated decay pathway [15]. Importantly, this Edc3p-mediated YRA1 pre-mRNA decay is dependent on the presence of the YRA1 intron and appears to require the function of the general mRNA export factor Mex67p [15]. Here, we have dissected the intronic decay element and the role of Mex67p in Edc3p-mediated YRA1 pre-mRNA decay. Our experiments delineated five structurally distinct but functionally interdependent cis-acting modules within the intron. Two modules dictate Edc3p substrate specificity and are designated as Edc3p responsive elements (ERE)s, whereas the other three modules, designated as translational repression elements (TRE)s, inhibit the translation of YRA1 pre-mRNA. This translational repression requires Mex67p and Mtr2p, but not Edc3p, and prevents YRA1 pre-mRNA from becoming a substrate for the NMD pathway.


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Abbreviations: AREs, adenine/uridine-rich elements; CTEs, constitutive transport elements; EREs, Edc3p responsive elements; eRF1, eukaryotic release factor 1; NGD, no-go decay; NMD, nonsense-mediated mRNA decay; NSD, non-stop decay; SC, synthetic complete; TREs, translational repression elements; UTR, untranslated region

* E-mail: feng.he@umassmed.edu

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YRA1 pre-mRNA Is Translationally Repressed by a Mechanism Independent of Edc3p Activity

YRA1 pre-mRNA contains multiple in-frame nonsense codons in its intron and, as such, could be considered to be a typical NMD substrate when present in the cytoplasm [18,20]. Nevertheless, upon export from the nucleus, this pre-mRNA is degraded by the Edc3p-mediated decay pathway, not the NMD pathway [15]. Since NMD is dependent on translation, the NMD resistance of YRA1 pre-mRNA could be attributable to translational repression of the transcript. Moreover, since YRA1 pre-mRNA is still largely resistant to NMD in the absence of Edc3p [15], the hypothetical translational repression mechanism must not require Edc3p. To assess these possibilities, we analyzed the translation status of YRA1 pre-mRNA in wild-type and edc3Δ strains utilizing sucrose gradient fractionation and northern blotting. In wild-type cells, the majority (70%) of the YRA1 pre-mRNA population was present in the mRNP fractions, with only modest representation (30%) in the polyribosome fractions. In contrast, most (75%) of the YRA1 mRNA was associated with the polyribosome fractions and only 25% was located in the mRNP fractions (Figure 1A). These results suggest that YRA1 pre-mRNA is indeed translationally repressed in wild-type cells, whereas YRA1 mRNA is not. Deletion of EDC3 did not affect the overall polyribosome profile or significantly alter the distribution of YRA1 pre-mRNA in the gradient. In edc3Δ cells, the
majority (64%) of pre-mRNA transcripts was still present in the mRNP fractions. Likewise, the distribution of YRA1 mRNA was essentially unaffected by deletion of EDC3 (Figure 1B). These results indicate that, in spite of its cytoplasmic localization [15], YRA1 pre-mRNA in wild-type or edc3Δ cells is translationally repressed, and Edc3p plays little role in establishing this repression.

Edc3p-Mediated YRA1 Pre-mRNA Degradation Does Not Require Translation

The observed translational repression of YRA1 pre-mRNA suggests that Edc3p-mediated YRA1 pre-mRNA degradation occurs independently of ongoing protein synthesis. To test this notion, we carried out two sets of experiments. First, we examined the effects of trans-inhibition of translation initiation, elongation, or termination on YRA1 pre-mRNA accumulation. In these experiments, initiation was inhibited by using the temperature-sensitive prtl-1 allele to inactivate Prt1p, a component of the translation initiation factor eIF3 complex [21]; elongation was inhibited by treating cultures with the drug cycloheximide [22]; and termination was inhibited by using the temperature-sensitive sup45-2 allele to inactivate Sup45p, the yeast eukaryotic release factor 1 (eRF1) [23]. We evaluated the effects of each of these three translation

Figure 2. Trans- and cis-inhibition of translation have no effect on Edc3p-mediated YRA1 pre-mRNA degradation. (A) Effects of trans-inhibition of translation on the steady-state levels of YRA1 pre-mRNA and mRNA. Initiation was inhibited by inactivation of Prt1p, termination was inhibited by inactivation of Sup45p, and elongation was inhibited by treating cells with cycloheximide. At the indicated times post-inhibition, RNA was isolated from culture aliquots and subjected to Northern analysis. Blots were hybridized with probes complementary to the YRA1, ADE2, or SCR1 transcripts, with the latter serving as a loading control. (B) The effects of cis-inhibition of translation initiation. A stem-loop structure was inserted into the 5′-UTRs of the YRA1 gene or its C-773 allele and the relative steady-state levels of the respective pre-mRNA and mRNA transcripts in wild-type (1), upf1Δ (2), edc3Δ (3), and upf1Δ.edc3Δ (4) cells were determined by Northern blotting as in (A). A schematic diagram of full-length YRA1 pre-mRNA and the related transcripts derived from the SL31-YRA1, C-773, and SL31-C-773 alleles is shown above the Northern blot. Smaller rectangles denote the 5′- and 3′-UTRs and larger rectangles denote the exons and the intron. The relative position of the 5′-UTR stem-loop structure is indicated, as are the nucleotides comprising the A of the initiator AUG (1), the 5′ (285) and 3′ (1052) boundaries of the intron, and the terminal nucleotide of the termination codon (1447).
blocks in both EDC3 and edc3A backgrounds and found that none of them affected the accumulation of YRA1 pre-mRNA. While subjected to any of these three translation blocks, EDC3 cells all accumulated low levels of YRA1 pre-mRNA and edc3A cells all accumulated high levels of YRA1 pre-mRNA (Figure 2A). As controls, we found that each of the three translation blocks caused 2- to 5-fold increases in the levels of nonsense-containing ade2-1 mRNA (Figure 2A). These results show that inhibition of any of the three basic steps of translation has no effect on the degradation of YRA1 pre-mRNA, i.e., even when general translation is inhibited YRA1 pre-mRNA is still degraded in an Edc3p-dependent manner.

We also examined whether inclusion of a cis-acting inhibitor of translation had any effect on YRA1 pre-mRNA decay. In this experiment, we inserted a stem-loop structure known to inhibit translation initiation [24] 31 nt upstream of the YRA1 initiator AUG and analyzed the steady-state levels of the resulting SL31-YRA1 pre-mRNA in wild-type, upf1Δ, edc3Δ, and upf1Δedc3Δ cells by northern blotting (the UPF1 gene encodes a key component of the NMD pathway). We found that the SL31-YRA1 pre-mRNA behaved like the wild-type pre-mRNA, i.e., it accumulated to low levels in wild-type or upf1Δ cells but increased 3- to 5-fold in edc3Δ or upf1Δedc3Δ cells (Figure 2B). As a control, we also inserted this stem-loop structure into the 5′-UTR of the yra1 C-773 allele. This allele contains a large 5′ deletion of the YRA1 intron and codes for a pre-mRNA that is degraded by NMD but not by Edc3p-mediated decay (Figure 2B). As expected, SL31-C-773 pre-mRNA was stabilized more than 5-fold in cells with wild-type NMD function (Figure 2B).

**Figure 3.** Effects of 5′ and 3′ deletions of the YRA1 intron on Edc3p-mediated YRA1 pre-mRNA decay. A set of yra1 alleles containing 3′ or 5′ deletions of the YRA1 intron was constructed and the steady-state levels of transcripts encoded by each of these alleles in wild-type (1), upf1Δ (2), edc3Δ (3), and upf1Δedc3Δ (4) cells were determined by Northern blotting. Blots were hybridized with probes complementary to the YRA1 or SCR1 transcripts, with the latter serving as a loading control. The positions of YRA1 pre-mRNAs encoded by the endogenous and all the exogenous YRA1 alleles are marked by a triangle and by diamonds, respectively. A schematic diagram of the yra1 alleles analyzed is shown above the Northern blot, with the relative position of each deletion indicated. Pre-mRNAs encoded by each of the YRA1 mutant alleles cannot be spliced to produce mRNAs, as the 5′ or 3′ splicing signals were deleted from these pre-mRNAs.

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These data show that a cis-acting inhibitor of translation also fails to affect the decay of the YRA1 pre-mRNA. Collectively, the experiments utilizing cis- or trans-inhibition of protein synthesis indicate that Edc3p-mediated YRA1 pre-mRNA degradation does not require concomitant translation of the transcript and suggest that translational repression may be an important component of Edc3p-mediated YRA1 pre-mRNA decay.

Edc3p-Mediated YRA1 Pre-mRNA Degradation Requires Multiple Elements of the YRA1 Intron

We previously showed that Edc3p-mediated YRA1 pre-mRNA degradation requires sequences within the YRA1 intron [15]. To delineate the pertinent cis-regulatory elements, we generated a set of YRA1 pre-mRNA transcripts harboring 3′ or 5′ deletions of the YRA1 intron and analyzed the effects of each of these deletions on the steady-state levels of YRA1 pre-mRNA in wild-type, upf1Δ, edc3Δ, and upf1Δedc3Δ cells. We included upf1Δ strains in these analyses because some intron deletions that inactivate Edc3p-mediated decay may simultaneously render a transcript sensitive to NMD, as each of these pre-mRNAs contains premature termination codons.

Deletions from the 3′ end of the intron yielded three distinct pre-mRNA decay phenotypes. First, deletions up to nt 742 (including alleles N-742 in Figure 3, and N-742, N-585, N-772, and N-732 in Figure S1) left the YRA1 pre-mRNA decay phenotype unchanged. Much like full-length wild-type YRA1 pre-mRNA, the pre-mRNA transcripts encoded by these alleles showed increased accumulation (at least 5-fold) only in edc3Δ or upf1Δedc3Δ cells (Figure 3), indicating that these transcripts are still degraded exclusively by the Edc3p-mediated pathway. Second, further deletions from nt 742 to nt 372 (including alleles N-712 and N-372 in Figure 3, and N-342 and N-400 in Figure S1) resulted in partial sensitivity to both NMD and Edc3p-mediated decay. The pre-mRNA transcripts encoded by these alleles showed modest increases (1.5- to 2-fold) in upf1Δ or edc3Δ strains but exhibited dramatic increases (more than 5-fold) in the upf1Δedc3Δ strain (Figures 3 and S1), indicating that these transcripts are degraded by either the Edc3p pathway or the NMD pathway. Since NMD requires translation, these results suggest that the region from nt 372 to 742 contains sequences that function in repressing YRA1 pre-mRNA translation, thus inhibiting its degradation by NMD. Finally, further deletions from nt 372 (including alleles N-311 in Figure 3 and N-342 in Figure S1) resulted in sensitivity only to NMD and Edc3p-mediated decay. The pre-mRNA transcripts encoded by these alleles showed modest increases (1.5- to 2-fold) in upf1Δ or edc3Δ strains but exhibited dramatic increases (more than 5-fold) in the upf1Δedc3Δ strain (Figures 3 and S1), indicating that these transcripts are degraded exclusively by the NMD pathway. These results suggest that sequences 5′ of nt 372 of the YRA1 intron are required for the Edc3p response. Since the smallest transcript capable of responding to Edc3p is that derived from construct N-372, which contains intron sequences from nt 286–372, this region must contain an ERE and we designate this segment as module A.

Deletions from the 5′ end of the intron up to nt 559 (including alleles C-559 in Figure 3 and C-395, C-343, C-348, and C-553 in Figure S2) did not affect the YRA1 pre-mRNA decay phenotype. All of the pre-mRNA transcripts encoded by these alleles are degraded exclusively by the Edc3p pathway. Further 5′-end deletions, from nt 559 to nt 672 of the intron (including alleles C-565 and C-672 in Figure 3 and C-625, C-637, C-648, and C-660 in Figure S2), eliminated the sensitivity to Edc3p-mediated decay. Interestingly, and in sharp contrast to the 3′ deletions described above, these deletions did not result in sensitivity to NMD. The pre-mRNA transcripts encoded by these alleles had 2- to 3-fold higher levels than full-length YRA1 pre-mRNA in wild-type cells but exhibited similar levels in all four strains (Figures 3 and S2). These results indicate that the region between nt 559-672 of the YRA1 intron is required for triggering Edc3p-mediated decay. This segment must also contain an ERE and we designate this region as module B.

Further 5′-end deletions, from nt 673 to 943 (including alleles C-943 in Figure 3 and C-678, C-683, C-713, C-773, and C-853 in Figure S2), resulted in sensitivity to Upf1p but not to Edc3p, indicating that these transcripts are primarily degraded by NMD. These results suggest that sequences downstream of nt 672 might function in repressing YRA1 pre-mRNA translation and thus inhibit the transcript’s degradation by NMD. Since the 3′ deletion analyses suggested that sequences upstream of nt 742 inhibit YRA1 pre-mRNA degradation by NMD, the region from nt 672–742 is most likely involved in repressing YRA1 pre-mRNA translation. Accordingly, we consider it to function as a TRE and designate this region as module C.

Taken together, these deletion analyses identified three intronic regions involved in the decay of YRA1 pre-mRNA: modules A and B are required for the Edc3p response and module C appears to function in repressing YRA1 pre-mRNA translation.

YRA1 Intron Modules Exhibit Synergistic and Partially Redundant Activities in Edc3p-Mediated YRA1 Pre-mRNA Decay

The results obtained with 3′ deletions of the intron implied that there is a functional dependency of ERE modules A and B on TRE module C, as a YRA1 pre-mRNA containing ERE modules A and B but lacking TRE module C is partially susceptible to NMD (allele N-712, Figure 3). Interestingly, the two ERE modules do not share significant sequence homology and appear to have some functional difference. YRA1 pre-mRNA containing module A alone is susceptible to both NMD and Edc3p-mediated decay (allele N-372, Figure 3). In contrast, YRA1 pre-mRNA containing module B alone exhibits exclusive substrate specificity for NMD (allele I-R4-NR2, Figure S3). These observations raise the possibility that ERE modules A and B may have different requirements for TRE elements and perform at least partially redundant functions in Edc3p-mediated YRA1 pre-mRNA decay. To assess this possibility, we generated YRA1 pre-mRNAs containing different combinations of intron modules A, B, C, or additional intronic sequences, and analyzed the steady-state levels of the transcripts encoded by these alleles in wild-type, upf1Δ, edc3Δ, and upf1Δedc3Δ cells. Transcripts containing modules A and C exhibited the same specificity for the Edc3p-mediated pathway as did full-length YRA1 pre-mRNA (allele R-AC, Figure 4). Transcripts containing modules B and C exhibited specificity for NMD but not for Edc3p-mediated decay (alleles R-BC, Figure 4). Notably, transcripts containing modules B and C plus downstream sequences up to nt 942 exhibited specificity for Edc3p-mediated decay comparable to that manifested by full-length YRA1 pre-mRNA (allele R-BCD, Figure 4). This result suggests that the segment downstream of module C, from nt 743 to nt 942, is also involved in Edc3p-mediated YRA1 pre-mRNA degradation and we, therefore, designate this region as module D. Altogether, these data show that the ERE modules A and B indeed collaborate with different TRE modules and have at least partially redundant activities in Edc3p-mediated YRA1 pre-mRNA decay.

Although the results described above implicated modules C and D in translational repression, transcripts containing modules C and D still exhibited specificity for NMD (allele R-CD, Figure 4).
In this experiment, the first transcript, C-672, is refractory to both Edc3p-mediated decay and NMD (Figure 3). (Figure 5A and 5B), contains intact modules C, D, and E and all designated as TREs. These results indicate that the respective ERE and C, or B, C, and D, is sufficient to trigger robust Edc3p-degradation by NMD. Importantly, a combination of modules A translation since these modules together can inhibit the transcript’s per se. Modules C, D, and E are not required for the Edc3p response functions. Modules A and B are required for the Edc3p response. As described below, modules C, D, and E can function together to repress YRA1 pre-mRNA translation and they were, therefore, all designated as TREs.

Taken together, these experiments indicate that YRA1 pre-mRNA degradation appears to involve five intronic sequence elements (Figure 4) and these five modules encompass two distinct functions. Modules A and B are required for the Edc3p response. Modules C, D, and E are not required for the Edc3p response per se but are most likely involved in repressing YRA1 pre-mRNA translation. We designated this region as module E. As described below, modules C, D, and E can function together to repress YRA1 pre-mRNA translation and they were, therefore, all designated as TREs.

However, transcripts containing modules C and D plus downstream sequences up to nt 1052 are refractory to NMD (allele R-CDE; Figure 4). This result suggests that the segment downstream of module D, from nt 942–1052, also plays a role in repressing YRA1 pre-mRNA translation. We designated this region as module E. As described below, modules C, D, and E can function together to repress YRA1 pre-mRNA translation and they were, therefore, all designated as TREs.

YRA1 Intron Modules C, D, and E Mediate Translational Repression of YRA1 Pre-mRNA

To further assess the role of modules C, D, and E in the translational repression of YRA1 pre-mRNA, we examined the translation status of pre-mRNAs that contain or lack these modules in wild-type or upf1Δ cells. We analyzed two transcripts in this experiment. The first transcript, C-672 pre-mRNA (Figure 5A and 5B), contains intact modules C, D, and E and is refractory to both Edc3p-mediated decay and NMD (Figure 3). The second transcript, C-773 pre-mRNA (Figure 5C), is almost identical to C-672 pre-mRNA except that it lacks module C and part of module D. This transcript is susceptible to NMD but not to Edc3p-mediated decay (Figure 2B). We analyzed the first transcript in wild-type and upf1Δ cells but the second transcript only in upf1Δ cells, because the second transcript is susceptible to NMD and has low abundance in wild-type cells. As shown in Figure 5A and 5B, the majority of the C-672 transcript in both wild-type and upf1Δ cells was present in the mRNP fractions (68% in wild-type cells and 78% in upf1Δ cells), with only modest representation in the polyribosome fractions (32% in wild-type cells and 22% in upf1Δ cells). In contrast, the majority (80%) of the C-773 transcript in upf1Δ cells was present in the polyribosome fractions, with only modest representation (20%) in the mRNP fractions (Figure 5C). As a control, we analyzed the translation status of SL31-C-773 pre-mRNA in upf1Δ cells. SL31-C-773 pre-mRNA is identical to C-773 pre-mRNA but harbors a stem-loop structure in its 5′-UTR that can inhibit translation initiation. In contrast to the C-773 transcript (Figure 5C), the majority (62%) of the SL31-C-773 transcript was present in the mRNP fractions, with only 38% in the polyribosome fractions (Figure 5D). This experiment shows that inclusion of a cis-inhibitory structure of translation initiation into the 5′-UTR of the C-773 transcript causes a sizeable portion of that mRNA to shift from the polyribosome fractions to the mRNP fractions. These data show that the C-672 transcript, which contains intact modules C, D, and E, is largely translationally repressed. In contrast, the C-773 transcript, which contains only a part of module D and the entirety of module E, is actively engaged in translation.
Cis and Trans Regulation of YRA1 pre-mRNA Decay

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C-672 pre-mRNA

C-672

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Absorbance at 254 nm

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22% 78%

C-672 pre-mRNA

C-672

1 2 3 4 5 6 7 8 9 10 11 12

C

upf1Δ

Absorbance at 254 nm

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80% 20%

C-737 pre-mRNA

C-737

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D

upf1Δ

Absorbance at 254 nm

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38% 62%

SL31-C-737 pre-mRNA

SL31-C-737

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E

HA-C-672

HA-C-737

Western blot analysis

Northern blot analysis

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F

YRA1

N-400

SL31-N-400

YRA1 pre-mRNA

N-400 YRA1 mRNA

SL31-N-400 YRA1 mRNA

SCR1 RNA

SCR1 RNA
To independently evaluate the above conclusion, we also constructed HA-tagged C-672 and C-773 yra1 alleles and analyzed their RNA and protein expression in wild-type, upf1Δ, edc3A, and upf1Δedc3A cells. The HA-C-672 allele showed the same RNA expression pattern as the untagged C-672 allele (compare allele HA-C-672 in Figure 5E to allele C-672 in Figure 3). The YRA1 pre-mRNA encoded by the HA-C-672 allele was insensitive to deletion of UPF1 or EDC3 and exhibited comparable high levels of expression in all four strains. Similarly, the HA-C-773 allele showed the same RNA expression pattern as the untagged C-773 allele (compare allele HA-C-773 in Figure 5E to allele C-773 in Figure 2B). The YRA1 pre-mRNA encoded by the HA-C-773 allele was sensitive to UPF1 but not to EDC3 and exhibited relatively low levels of expression in wild-type and edc3A cells but high levels in upf1Δ and upf1Δedc3A cells. However, despite the high levels of accumulation of the HA-C-672 pre-mRNA in wild-type, upf1Δ, edc3A, and upf1Δedc3A cells, no protein expression was detected from this transcript (Figure 5E). In contrast, protein expression was detected from the HA-C-773 pre-mRNA in all four strains (Figure 5E). Collectively, these experiments show that YRA1 intron modules C, D, and E function in repressing YRA1 pre-mRNA translation and suggest that this translational repression activity requires the combined actions of all three modules.

Our observation that intron modules C, D, and E function in repressing YRA1 pre-mRNA translation further indicates that translational repression is a critical component of Edc3p-mediated YRA1 pre-mRNA decay. However, the complexity of the YRA1 intron elements and their functional interaction patterns raise the question of whether modules C, D, and E are only involved in translational repression or have additional regulatory functions (e.g., Edc3p substrate specificity). To address this issue, we tested whether cis-inhibition of translation initiation can suppress the defect caused by deletion of modules C, D, and E. In this experiment, we used the module A-containing N-400 allele (Figure 5F). We inserted a stem-loop structure 31 nt upstream of the initiator AUG codon of the N-400 allele to generate the SL31-N-400 allele (Figure 5F) and analyzed the decay phenotype of the transcripts encoded by this allele in wild-type, upf1Δ, edc3A, and upf1Δedc3A cells. Unlike the N-400 pre-mRNA, which is a partial Edc3p substrate, the SL31-N-400 pre-mRNA behaved like a bona fide Edc3p substrate (Figure 5F). These data show that cis-inhibition of translation initiation suppresses the defect caused by complete deletion of modules C, D, and E and fully restores the substrate status of an otherwise partial Edc3p substrate. These results indicate that YRA1 intron modules C, D, and E function principally in repressing YRA1 pre-mRNA translation and do not contribute to Edc3p substrate specificity directly.

Mex67p is a Component of the Cytoplasmic YRA1 Pre-mRNA Decay and Functions in Repressing YRA1 Pre-mRNA Translation to Enhance Edc3p-Mediated Decay

We previously found that inactivation of Mex67p triggers rapid degradation of the YRA1 pre-mRNA by NMD in edc3A cells [15]. Since NMD requires translation while Edc3p-mediated decay does not, we considered the possibility that Mex67p is a determinant of YRA1 pre-mRNA translational repression. To assess the role of Mex67p in translational repression, we first analyzed the effect of inactivation of Mex67p on translation of YRA1 pre-mRNA in upf1Δedc3ΔAmex67-5 cells. As shown in Figure 6A, when cells were grown at the permissive temperature (25°C), a significant fraction of YRA1 pre-mRNA is located in the mRNP fractions. However, when cells were shifted to the non-permissive temperature (37°C) for just 6 min, YRA1 pre-mRNA originally located in the mRNP fractions moved to the polysome fractions. Importantly, this temperature shift did not cause the redistribution of the YRA1 mRNA over the sucrose gradient. As a control, we also analyzed the translation status of YRA1 pre-mRNA in upf1Δedc3ΔAmex67-5 cells. We found that YRA1 pre-mRNA in either the mRNP fractions or the polysome fractions remains unchanged during this 6-min temperature shift (Figure S4). These data show that inactivation of Mex67p mitigates the translational repression of YRA1 pre-mRNA and suggest a direct role of Mex67p in translational control of YRA1 pre-mRNA.

The apparent involvement of Mex67p in repressing YRA1 pre-mRNA translation suggested that Mex67p binds to the YRA1 pre-mRNA. To test this possibility, we constructed a yeast upf1Δedc3Δ strain harboring a HA-tagged Mex67p allele and analyzed the association of Mex67p with YRA1 pre-mRNA in this strain by co-immunoprecipitation and RT-PCR assays. As shown in Figure 6B, using anti-HA monoclonal antibodies, we were able to precipitate about 80% of HA-Mex67p fusion protein from whole-cell extracts and observed significant enrichment for YRA1 pre-mRNA in the HA-Mex67p pellet. This result implies that Mex67p binds to YRA1 pre-mRNA and is a component of the YRA1 pre-mRNP.

To further address the role of Mex67p in translation repression of YRA1 pre-mRNA, we tested whether tethering this protein can inhibit YRA1 pre-mRNA degradation by NMD. In our initial experiment, we inserted two tandem MS2 coat protein binding sites into the intronic regions immediately upstream of module D...
Figure 6. Mex67p is a component of the cytoplasmic YRA1 pre-mRNP that functions in repressing YRA1 pre-mRNA translation. (A) Analysis of the effect of inactivation of Mex67p on the translation of YRA1 pre-mRNA. upf1Δ edc3Δ mex67-5 cells were grown at 25°C and then shifted to 37°C for 6 min. The polyribosomal association of YRA1 pre-mRNA and mRNA in these cells before or after the temperature shift was analyzed by sucrose gradient fractionation and Northern blotting. Upper panels: absorbance tracings at 254 nm; lower panels: Northern blots of individual gradient fractions. Blots were hybridized with a probe complementary to YRA1 transcripts. The percentages of YRA1 pre-mRNA and mRNA in the mRNP and the polyribosomal fractions are indicated. (B) Analysis of the association of Mex67p with YRA1 pre-mRNA. Whole cell extracts from upf1Δ edc3Δ strains harboring either the MEX67 or the HA-MEX67 allele were incubated with anti-HA antibodies. Proteins and RNAs precipitated by the
sequences in the F7 and R1-F7 alleles and then analyzed the steady-state levels of the transcripts encoded by the resulting F7-MS2 and R1-F7-MS2 alleles in wild-type, upf1Δ, edc3Δ, and upf1Δedc3Δ cells which either express or do not express the MS2 coat protein/Mex67p fusion protein (MS2-coat-Mex67p). The R1-F7 allele is almost identical to the F7 allele except that it lacks a functional module A. The pre-mRNA transcript encoded by the F7 allele is a bona fide NMD substrate (Figure S5). In contrast, the pre-mRNA transcript encoded by the R1-F7 allele is a bona fide NMD substrate (Figure S5). In cells which did not express MS2-coat-Mex67p, the F7-MS2 and R1-F7-MS2 transcripts behaved the same as their counterpart transcripts lacking the MS2-binding sites (Figure 6C and Figure S5). The F7-MS2 transcript was a substrate for both NMD and Edc3p and the R1-F7-MS2 transcript was a substrate for NMD (Figure 6C). However, in cells which expressed MS2-coat-Mex67p, the F7-MS2 transcript dramatically changed its decay phenotype. This transcript was no longer a substrate for NMD and behaved like a bona fide Edc3p substrate (Figure 6C). In contrast, the R1-F7-MS2 transcript did not change its decay phenotype and maintained its status as an NMD substrate (Figure 6C). As controls, we also analyzed the decay phenotypes of F7-MS2 and R1-F7-MS2 pre-mRNAs in cells which expressed MS2 coat protein/Sub2p, Edc3p, or Crr1p fusion proteins. We found that the decay phenotypes of the F7-MS2 and R1-F7-MS2 transcripts remained unchanged in cells which expressed each of these fusion proteins (Figure 6C and unpublished data). These results show that tethering of Mex67p inhibits degradation of the transcript by NMD yet promotes its degradation by Edc3p-mediated decay. However, this NMD-inhibitory effect of tethering Mex67p is dependent on at least ERE module A.

To determine whether the NMD-inhibitory effect of tethering Mex67p requires additional cis elements besides the module A ERE, we analyzed two additional intron-containing yra1 alleles, F12-MS2 and N-400-MS2. These two alleles are identical to the F7-MS2 allele except that F12-MS2 lacks module D and N-400-MS2 lacks both modules D and E. In contrast to the F7-MS2 transcript, in cells which expressed MS2-coat-Mex67p, the transcripts encoded by the F12-MS2 or N-400-MS2 alleles remained as substrates for both NMD and Edc3p (Figure 6C). These results show that tethering Mex67p neither inhibited the degradation by NMD nor promoted the degradation by Edc3p for either of these transcripts. Taken together, our data show that tethering Mex67p to YRA1 pre-mRNA can inhibit its translation and this inhibitory effect requires at least intron modules A and D.

We also find that, at least in edc3Δ cells, YRA1 pre-mRNA localized to the polyribosome fractions co-sedimented with YRA1 mRNA (Figure 1B), a surprising result that was also observed in additional experiments described in Figure 6A and Figure S4. Since YRA1 pre-mRNA has a much shorter coding region than YRA1 mRNA (285 nt versus 678 nt), these observations suggest that translationally repressed mRNPs have unusual compositions or conformations that may reflect blocks to more than one step in translation.

Inactivation of Mtr2p Results in YRA1 Pre-mRNA Degradation by NMD

The results described in Figure 6 show that Mex67p plays a role in repressing YRA1 pre-mRNA translation, inhibiting YRA1 pre-mRNA degradation by NMD, and promoting the transcript’s degradation by Edc3p. Since Mex67p and Mtr2p function as a complex in mRNA export [25], we sought to assess whether Mtr2p also plays a role in YRA1 pre-mRNA decay. Accordingly, we constructed a set of edc3Δ and upf1Δedc3Δ strains harboring temperature-sensitive mtr2 alleles and analyzed the effect of Mtr2p inactivation on the accumulation of YRA1 pre-mRNA. We analyzed three different temperature-sensitive alleles (mtr2–9, mtr2–21, and mtr2–26) and included the fully functional GFP-tagged MTR2 allele as a wild-type control. Previous studies had shown that the proteins encoded by these three temperature-sensitive alleles no longer interacted with Mex67p and at the restrictive temperature, cells harboring each of these alleles all manifested inhibition of nuclear mRNA export and mislocalization of Mex67p to cytoplasmic foci [25]. Our analyses indicated that, at the permissive temperature (25°C), edc3Δ cells harboring the GFP-MTR2, mtr2–9, mtr2–21, or mtr2–26 alleles all accumulated comparably high levels of YRA1 pre-mRNA (compare “0” time points, left panels in Figure 7A–D). However, when shifted to the restrictive temperature (37°C), edc3Δ cells harboring the GFP-MTR2 allele behaved dramatically different from edc3Δ cells harboring the mtr2–9, mtr2–21, or mtr2–26 alleles. During the 24-min time course, edc3ΔGFP-MTR2 cells maintained relatively high levels of YRA1 pre-mRNA at each time point (Figure 7A, left panel). In contrast, edc3Δ cells harboring the mtr2–9, mtr2–21, or mtr2–26 alleles exhibited significant decreases in YRA1 pre-mRNA level for each time point (compare the 3 6 12 and 24-min time points, Figure 7B–D left panels to that of Figure 7A). These decreases were transcript-specific since, during the 24-minute time course, PGR1 mRNA levels remained unchanged in edc3Δ and edc3Δupf1Δ cells harboring the GFP-MTR2, mtr2–9, mtr2–21, or mtr2–26 alleles (Figures 7A–D). Moreover, the dramatic decrease of YRA1 pre-mRNA levels in edc3Δ mtr2 cells was likely caused by rapid degradation of YRA1 pre-mRNA by NMD, especially at the early time points (3 and 6 min), because deletion of UPF1 mitigated these decreases (Figure 7B–D, right panels). Deletion of UPF1 from edc3Δamb2–9, edc3Δamb2–21, and edc3Δ mtr2–26 cells did not result in increased YRA1 pre-mRNA accumulation at late time points (12 and 24 min, Figure 7B–D, right panels), suggesting that inactivation of Mtr2p may have also blocked YRA1 pre-mRNA nuclear export, causing almost complete depletion of the cytoplasmic pool of YRA1 pre-mRNA. Consistent with this interpretation, mtr2–9, mtr2–21, or mtr2–26 cells in both edc3Δ and edc3Δupf1Δ backgrounds all accumulated a new, longer YRA1 mRNA species at late time points of the temperature shift (12 and 24 min, Figure 7B–D). The accumu-
Figure 7. Inactivation of Mrt2p causes rapid degradation of YRA1 pre-mRNA by NMD. edc3Δ and edc3Δupf1Δ cells harboring the fully functional GFP-MTR2 (A) allele or the temperature-sensitive mtr2–9 (B), mtr2–21 (C), or mtr2–26 (D) alleles were grown at the permissive temperature (25°C), then shifted to the restrictive temperature (37°C) for indicated times. Cells from each time point were collected and the levels of YRA1 or PGK1 transcripts were analyzed by Northern blotting. Blots were hybridized with probes complementary to the YRA1, PGK1, or SCR1 transcripts, with the latter serving as a loading control. The positions of the normal YRA1 mRNA species and the atypical longer YRA1 mRNA species that accumulated in cells harboring the mtr2–9, mtr2–21, or mtr2–26 alleles at late time points are indicated by a triangle and by diamonds, respectively. Graphs to the right of the figure depict YRA1 pre-mRNA levels for each allele +/− Upf1p normalized to the corresponding “0” time point.
doi:10.1371/journal.pbio.1000360.g007
loration of this new YRA1 mRNA species is reminiscent of our previous observations in cells subject to Mex67p inactivation [15]. Taken together, the consequences of Mrtp2p inactivation suggest that, similar to its role in general mRNA export, Mrtp2p likely functions in a complex with Mex67p to repress YRA1 pre-mRNA translation.

Discussion

Edc3p-mediated YRA1 pre-mRNA decay occurs in the cytoplasm [15]. In contrast to other cytoplasmic decay pathways, such as the NMD and general 5′ to 3′ decay pathways [1,2], our data indicate that Edc3p-mediated YRA1 pre-mRNA decay occurs independently of translation. This conclusion is supported by several observations, notably: (1) YRA1 pre-mRNA is in a translationally repressed state in wild-type and edc3Δ cells (Figure 1); (2) trans-inhibition of general translation initiation, elongation, or termination has no significant effect on the steady-state levels of YRA1 pre-mRNA in both Edc3 and edc3Δ backgrounds (Figure 2A); and (3) inclusion of a cis-inhibitor of translation initiation in the 5′-UTR of YRA1 pre-mRNA also has no effect on its decay (Figure 2B). Our finding that Edc3p-mediated YRA1 pre-mRNA decay does not involve translation explains why this transcript is largely resistant to NMD despite the fact that it resembles a typical NMD substrate [20] and suggests that translational repression is a critical component of Edc3p-mediated YRA1 pre-mRNA decay.

Our deletion analyses revealed five distinct modules important for Edc3p-mediated YRA1 pre-mRNA decay. Modules A and B are required for triggering an Edc3p response and are thus bona fide EREs (Figures 3 and 4). Modules C, D, and E are required for inhibiting translation and function as TREs (Figures 4 and 5). Significantly, each of these modules except module A lacks an independent activity and appears to function synergistically with other modules essential for Edc3p-mediated YRA1 pre-mRNA degradation (Figures 3, 4, and S3). Functional synergy was indeed manifested in several combinations of these modules. A combination of TRE modules C, D, and E can repress YRA1 pre-mRNA translation (Figures 4 and 5) and two different combinations of ERE and TRE modules can trigger efficient Edc3p-mediated YRA1 pre-mRNA decay (Figure 4). The underlying molecular mechanisms for these synergistic effects are currently not clear but may be indicative of cooperative binding to these intron modules by either different factors or different domains of the same factor. Interestingly, the two ERE modules (A and B) appear to lack synergistic activities and also have different functional requirements for TREs (Figures 4 and S6), suggesting that these two ERE modules perform at least partially redundant functions in Edc3p-mediated YRA1 pre-mRNA decay (Figures 4 and S7).

Our observation that the YRA1 intron contains two distinct functional elements indicates that the intron modules perform at least two essential functions in Edc3p-mediated YRA1 pre-mRNA decay. The EREs appear to dictate Edc3p substrate specificity and most likely function to recruit Edc3p to the YRA1 pre-mRNA. The TREs repress YRA1 pre-mRNA translation and thus prevent degradation of the pre-mRNA by translation-dependent NMD and enhance its degradation by Edc3p. Since elimination of both EREs does not have any significant effects on TRE-mediated translational repression of YRA1 pre-mRNA (Figure 5), and in contrast, the elimination of the three TREs causes partial loss of the ERE-mediated Edc3p response (Figure 3), these observations suggest that TRE-mediated translational repression functions upstream of ERE-mediated recruitment of Edc3p. When combined with the fact that Edc3p interacts with the Dcp1/Dcp2 decapping enzyme [26–33], these functional data indicate that Edc3p-mediated YRA1 pre-mRNA degradation is most likely carried out through a series of ordered events including translational repression, recognition by Edc3p, recruitment of the decapping enzyme, and finally activation of decapping.

The complex cis-regulatory elements involved in Edc3p-mediated YRA1 pre-mRNA decay are reminiscent of the localization elements identified in numerous mRNAs including ASH1 mRNA in the yeast Saccharomyces cerevisiae [34], nano mRNA in the fly Drosophila melanogaster [35,36], and Vg1 mRNA in the frog Xenopus laevis [37]. Like the YRA1 intronic decay element, mRNA localization elements for each of these transcripts consist of multiple functionally distinct modules that can function independently but can also act synergistically to ensure proper mRNA localization. Importantly, translational repression is also an integral part of a localization element’s function [34]. These observations suggest that mRNA decay may share common regulatory mechanisms with mRNA localization. Paradoxically, the YRA1 intronic decay element bears no similarity to the cis-element regulating decay of the RPS28B mRNA, the only other known Edc3p substrate [14]. The cis-element of RPS28B mRNA is localized in its 3′-UTR and appears to form a single stem-loop structure that binds Rps28p and recruits Edc3p [14]. These observations suggest that decay mechanisms may be significantly different even when transcripts are regulated by the same decay factor.

Several of our experiments demonstrate that the general mRNA exporter Mex67p is involved in repressing YRA1 pre-mRNA translation. First, inactivation of Mex67p alleviates translational repression of YRA1 pre-mRNA in edc3Δdup1A cells (Figure 6A). Second, Mex67p binds to YRA1 pre-mRNA (Figure 6B). Third, tethering Mex67p to an NMD-susceptible partial Edc3p substrate inhibits the transcript’s degradation by NMD and promotes its degradation by Edc3p-mediated decay (Figure 6C). Since the elimination of the TREs and inactivation of Mex67p have similar consequences on YRA1 pre-mRNA decay, i.e., increased susceptibility to NMD and diminished susceptibility to Edc3p-mediated decay, one interesting possibility is that Mex67p binds directly to at least one of the TREs. In support of this idea, our experiments revealed that the NMD-inhibitory effect of tethering Mex67p also requires TRE module D besides ERE module A (Figure 6C). Interestingly, the human homolog of Mex67p, Tap, is also a sequence-specific RNA-binding protein that binds directly to constitutive transport elements (CTEs) of both viral and cellular intron-containing mRNAs [38–40]. Similar to its role in general mRNA export, Mex67p likely functions as a complex with Mtr2p in repressing YRA1 pre-mRNA translation. Our finding that inactivation of Mex67p and Mtr2p both cause rapid degradation of YRA1 pre-mRNA by NMD (Figure 7 and [15]) lends strong support for this conclusion.

A role for the Mex67p/Mtr2p general mRNA export factors in repressing YRA1 pre-mRNA translation is intriguing and raises the possibility that these factors may also have a role in the translational control of additional mRNAs in yeast and in other eukaryotic cells. Consistent with this notion, the human homologues of Mex67p/Mtr2p (Tap/p15) have been shown to promote the translation of a CTE-containing mRNA [41] and the general yeast mRNA export factor, Gle1p, appears to interact with both translation initiation and termination factors and to regulate two distinct stages of translation [42]. Additionally, the general yeast mRNA export factor Dhp5p also exhibits genetic as well as physical interactions with translation termination factors [43]. These observations highlight the interconnections between different steps of the eukaryotic gene expression pathway and suggest that mRNA export factors may have a general as well as a
specific role in controlling cytoplasmic mRNA translation and decay.

**Materials and Methods**

**General Procedures**
Most strains, protocols, and materials used in this study have been described previously [15]. Additional procedures used herein are summarized below.

**Yeast Strains**
All strains used in this study are listed in Table S1. Strains containing deletions of EDC3 or UPF1 were constructed by gene replacement [44], using DNA fragments harboring the corresponding null alleles. Each genomic DNA deletion was confirmed by PCR analysis. Strains harboring the temperature-sensitive prtl-1 or upf45-2 alleles were constructed by the pop-in and pop-out technique [44]. Strains harboring the GFP-MTR2 allele or the temperature-sensitive mtr2-9, mtr2-21, and mtr2-26 alleles were constructed by plasmid shuffling [44].

**Plasmids**
All plasmids used in this study are listed in Table S2. YRA1 alleles harboring deletions of intron sequences, or containing insertions of a stem-loop structure, were generated by PCR and molecular cloning. YRA1-MS2 constructs were prepared by annealing two oligonucleotides containing two tandem MS2-coat protein recognition sites and inserting the resulting DNA fragment into the BamHI and EcoRI sites in the intronic region of the F7 and R1-F7 YRA1 alleles. All YRA1 alleles were confirmed by DNA sequencing. Plasmids expressing the MS2-coat protein fusions with GFP-MTR2, HA-C-672, and HA-C-773 constructs were prepared by molecular cloning. In each case, a DNA fragment harboring the coding and 5′-UTR sequences of MEX67 or SCB2 was amplified using a pair of oligonucleotides that contain the NheI (5′ primer) and SalI (3′ primer) sites. The resulting DNA fragment was then inserted between the NheI and SalI sites of a plasmid that contains the ADH1 promoter and the MS2 coding sequence. Each of these fusion proteins was expressed in vivo under the control of the ADH1 promoter. HA-tagged MEX67 allele was constructed by PCR and contains 410 bp from the promoter region of MEX67, 96 bp of triple HA, and 1,797 bp coding region and 260 bp 3′-UTR of MEX67. HA-tagged YRA1 C-672 and C-773 alleles were constructed by PCR. These two alleles are the same as their untagged alleles except that they contain a 101 bp Nol-NheI fragment encoding a triple HA tag inserted at the initiation codon. The HA-C-672 allele contains an in-frame stop codon six codons into the intron and HA-C-773 contains an in-frame stop codon at the exon-intron junction.

**Oligonucleotides**
The oligonucleotides used in this study were obtained from Operon, Inc., and are listed in Table S3.

**RNA Analysis**
Cells were grown in either YEPD (Figures 2A and S8), or synthetic complete (SC) medium lacking tryptophan (Figures 2B, 3, 4, 5E, 5F, 6B, S1, S2, S3, S5, S6, and S7), leucine (Figures 7), or leucine and tryptophan (Figure 6C). For normal cell cultures, cells (15 ml) were grown at 30°C to an OD 600 of 0.7 and harvested by centrifugation. For cultures involving cycloheximide treatment, cells (100 ml) were grown at 30°C to an OD 600 of 0.7, harvested by centrifugation, and resuspended in 20 ml of the same medium. Cycloheximide was added to cell cultures at a final concentration of 100 μg/ml and 2 ml of cell cultures were harvested at different time points. For cultures involving temperature shifts, cells were grown at 25°C and treated as described previously [43]. In each case, cell pellets were frozen on dry ice and then stored at −80°C until RNA isolation. The procedures for RNA isolation and northern blotting were as previously described [45]. Transcript-specific signals were determined with a FUJI BAS-2500 analyzer.

**Protein Analysis**
Preparation of whole-cell extracts and Western blotting procedures were as described previously [15]. Blots were probed with monoclonal antibodies against the HA epitope (12CA5, Roche) or against Pgp1p (22C5-D8, Molecular Probes), with the latter polypeptide serving as a loading control. Proteins were detected using ECL Western blotting detection reagents (GE Healthcare) and Kodak BioMax film.

**Polyosme Analysis**
Cells were grown at 30°C in either YEPD medium (Figure 1) or SC medium lacking tryptophan (Figures 5A-D, 6A, and S4) to an OD 600 of 0.7. Cell extracts were prepared and fractionated on 7%–42% sucrose gradients as previously described [46].

**Analysis of RNAs Associated with Mex67p**
Cells harboring an HA-tagged MEX67 allele (100 ml) were grown at 30°C in SC medium lacking tryptophan to an OD 600 of 0.7. Cells were collected by centrifugation, washed with ice-cold water, and resuspended in 1 ml of buffer B100 [10 mM Tris–HCl [pH 7.5], 100 mM KCl, 5 mM MgCl2, 1 mM dithiothreitol [DTT], 1 mM phenylmethylsulfonylfluoride, 10 mM Vanadyl ribonucleoside complex [VRC], 0.1% Nonidet P-40 [NP-40], 100 U/ml RNasin, and 1 μg protease inhibitor cocktail [Roche]. Cells were broken mechanically with glass beads and 50 μl extracts were incubated with 12 μl of a slurry of anti-HA agarose beads (Pierce) for 6 h at 4°C. The beads were washed four times with buffer B150 (same as B100 except containing 150 mM KCl). RNA from the input extract and the supernatant and pellet fractions of the immunoprecipitation was isolated by phenol/chloroform extraction and ethanol precipitation. RNA from each of these samples was treated with DNase I and then analyzed by RT-PCR using a cDNA synthesis kit from Roche. Portions (1/25) of the input extract and the supernatant and pellet fractions were also analyzed by Western blotting to evaluate the efficiency of immunoprecipitating HA-Mex67p.

**Supporting Information**

Figure S1 Effects of 3′ deletions of the YRA1 intron on Ede3p-mediated YRA1 pre-mRNA decay. A panel of yra1 alleles containing deletions from the 3′-end of the YRA1 intron was constructed and the steady-state levels of transcripts encoded by each of these alleles in wild-type (1), yra1A (2), edc3A (3), and yra1A edc3A (4) cells were determined by Northern blotting. Blots were hybridized with probes complementary to the YRA1 or SCR1 transcripts, with the latter serving as a loading control. The positions of YRA1 pre-mRNAs encoded by the endogenous and all the exogenous YRA1 alleles are marked by a triangle and by diamonds, respectively. A schematic diagram of the analyzed yra1 alleles is shown above the Northern blot, with the relative position of each deletion indicated. Pre-mRNAs encoded by each of the YRA1 mutant alleles cannot be spliced to produce mRNAs, as the 3′ splicing signals were deleted from these pre-mRNAs. The
transcripts are divided into three groups by broken lines based on their distinct decay phenotypes manifested in the Northern blots. 

**Figure S2** Effects of 5’ deletions of the YRA1 intron on Edc3p-mediated YRA1 pre-mRNA decay. A panel of yra1 alleles containing deletions from the 5’-end of the YRA1 intron was constructed and the steady-state levels of transcripts encoded by each of these alleles in wild-type (1), upf1A (2), edc3A (3), and upf1Aedc3A (4) cells were determined by Northern blotting. Blots were hybridized with probes complementary to the YRA1 or SCR1 transcripts, with the latter serving as a loading control. The positions of YRA1 pre-mRNAs encoded by the endogenous and all the exogenous YRA1 alleles are marked by a triangle and by diamonds, respectively. A schematic diagram of the analyzed yra1 alleles is shown above the Northern blot, with the relative position of each deletion indicated. Pre-mRNAs encoded by each of the YRA1 mutant alleles cannot be spliced to produce mRNAs, as they lack both the 5’ splicing signals were deleted from these pre-mRNAs. The transcripts are divided into three groups by broken lines based on their distinct decay phenotypes manifested in the Northern blots. 

Pre-mRNAs encoded by both alleles can produce mRNAs because they still contain all the necessary splicing signals. 

**Figure S3** Intronic modules B and C lack independent activity in Edc3p-mediated YRA1 pre-mRNA decay. A set of yra1 alleles containing different internal fragments of module B and C regions of the YRA1 intron was constructed and steady-state levels of the YRA1 pre-mRNA encoded by each of these alleles in wild-type (1), upf1A (2), edc3A (3), and upf1Aedc3A (4) cells were determined by Northern blotting. Blots were hybridized with probes complementary to the YRA1 intron modules A, B, C, D, and E indicated. Pre-mRNAs encoded by each of these recombinant YRA1 alleles can produce mRNAs as they still contain all the necessary splicing signals. 

**Figure S4** A temperature shift does not alter the translation status of YRA1 pre-mRNA in mex67Δ cells. Total RNA was isolated from wild-type (1), upf1A (2), edc3A (3), and upf1Aedc3A (4) and the steady-state levels of the YRA1 pre-mRNA in these cells were determined by Northern blotting. The blot was hybridized with probes complementary to the YRA1 intron modules A, B, C, D, and E indicated. Pre-mRNAs encoded by each of these recombinant YRA1 alleles can produce mRNAs because they still contain all the necessary splicing signals. 

**Figure S5** Analysis of the decay phenotypes of the YRA1 pre-mRNA transcripts encoded by the yra1 f7 and r1-f7 alleles. yra1 alleles harboring intrinsic deletions from nt 400 to 777 (f7) or from nt 311 to 777 (r1-f7) were constructed and the steady-state levels of the YRA1 pre-mRNAs encoded by these alleles in wild-type (1), 777 (f7) or from nt 311 to 777 (r1-f7) were constructed and the steady-state levels of the YRA1 pre-mRNAs encoded by these alleles in wild-type (1), upf1A (2), edc3A (3), and upf1Aedc3A (4) cells were determined by Northern blotting. The blot was hybridized with probes complementary to the YRA1 or SCR1 transcripts, with the latter serving as a loading control. The positions of YRA1 pre-mRNAs encoded by the endogenous and the exogenous alleles are marked by a triangle and by diamonds, respectively. A schematic diagram of the f7 and r1-f7 alleles analyzed is shown above the Northern blot, with the relative position of each deletion indicated. 

Pre-mRNAs encoded by both alleles can produce mRNAs because they still contain all the necessary splicing signals. 

**Figure S6** ERE module A does not exhibit functional interaction with TRE modules D and E. A set of yra1 alleles containing different combinations of YRA1 intron modules A, D, and E was constructed and the steady-state levels of transcripts encoded by each of these alleles in wild-type (1), upf1A (2), edc3A (3), and upf1Aedc3A (4) cells were determined by Northern blotting. Blots were hybridized with probes complementary to the YRA1 intron module A. The positions of YRA1 pre-mRNAs encoded by the endogenous and all the exogenous YRA1 alleles are marked by a triangle and by diamonds, respectively. A schematic diagram of the yra1 alleles analyzed is shown above the Northern blot, with the relative positions of modules A, B, C, D, and E indicated. Pre-mRNAs encoded by each of these recombinant YRA1 alleles can produce mRNAs as they still contain all the necessary splicing signals. 

**Figure S7** Deletion of either module A or B does not affect Edc3p-mediated YRA1 pre-mRNA decay. A set of yra1 alleles harboring deletions of either module A or B was constructed and the steady-state levels of the YRA1 pre-mRNAs encoded by each of these recombinant alleles in wild-type (1), upf1A (2), edc3A (3), and upf1Aedc3A (4) cells were determined by Northern blotting. Blots were hybridized with probes complementary to the YRA1 or SCR1 transcripts. The positions of YRA1 pre-mRNAs encoded by the endogenous and all the exogenous YRA1 alleles are marked by a triangle and by diamonds, respectively. A schematic diagram of the yra1 alleles analyzed is shown above the Northern blot, with the relative position of each deletion indicated. Pre-mRNAs encoded by each of these recombinant YRA1 mutant alleles can produce mRNAs because they still contain all the necessary splicing signals. 

**Figure S8** Deletion of UPF1 causes increased accumulation of YRA1 pre-mRNA in edc3A Δ cells. Total RNA was isolated from wild-type (1), upf1A (2), edc3A (3), and upf1Aedc3A (4) and the steady-state levels of the YRA1 pre-mRNA in these cells were determined by Northern blotting. The blot was hybridized with probes complementary to the YRA1 or SCR1 transcripts, with the latter serving as a loading control. The positions of YRA1 pre-mRNA and mRNA are indicated. 

**Table S1** Yeast strains used in this study. 

**Table S2** Plasmids used in this study. 

**Table S3** Oligonucleotides used in this study. 

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**Author Contributions**

The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: SD FH. Performed the experiments: SD FH. Analyzed the data: SD AJ FH. Contributed reagents/materials/analysis tools: SD FH. Wrote the paper: SD AJ FH.
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