Supplemental Information to:

Co-translational mRNA decay in *Saccharomyces cerevisiae*

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Supplemental Material

Endogenous decapped mRNAs are ribosome bound.

To demonstrate an interaction between the endogenous decapped mRNA and ribosomes, we purified polyribosomes from \textit{xrn1A} cells with an epitope-tagged 60S subunit\textsuperscript{9}. Primer extension for endogenous \textit{CYH2} and \textit{ADH1} mRNAs in the purified polyribosomes indicated that the decapped species from these two mRNAs can be pulled down by ribosomes (Fig. S3c), confirming they are associated with polyribosomes. The purification was specific; mRNA was not pulled down in the untagged control. Moreover the U1 snRNA was not detected in the purified polyribosomes (Fig. S3c).

\textit{PGK1\textsuperscript{RC}} mRNA polyribosome-associated decay fragments are truncated from the 5’ end.

The \textit{PGK1\textsuperscript{RC}} reporter accumulates decay fragments that correlate in size to ribosome occupancy (Fig. 3b). Since these decay intermediates can only be detected when the Northern probe is specific to the 3’UTR of \textit{PGK1\textsuperscript{RC}} (Fig. 3b), it suggests that decay fragment heterogeneity is the result of 5’ exonuclease digestion. To test this, we performed Northern blot analysis using probes spanning \textit{PGK1\textsuperscript{RC}} mRNA. Consistent with the prediction, the ~500nt fragment in the 80S fraction was not visible when a probe upstream of the rare codon stretch was used in Northern analysis (probe 2; Fig. S5). In addition, only the fragments in the heavier polyribosome regions could be detected when a 5’ end probe (probe 1) was used (Fig. S5). These results reveal that the heterogeneity of the fragments comes from the 5’ end of the reporter.

\textit{PGK1\textsuperscript{RC}} mRNA polyribosome-associated decay fragments are generated by the normal decay pathway.
We determined if the \textit{PGK1} \textit{RC} mRNA polyribosome-associated decay fragments (Fig. 3b) require decapping and 5\textsuperscript{-}3\textsuperscript{-} exonuclease digestion. The \textit{PGK1} \textit{RC} reporter was expressed in a decapping enzyme mutant (\textit{dcp2}Δ) and a 5\textsuperscript{-}3\textsuperscript{-} exonuclease mutant (\textit{xrn1}Δ). Using sucrose gradients, \textit{PGK1} \textit{RC} mRNA was analyzed in \textit{dcp2}Δ and \textit{xrn1}Δ cells. Importantly, ribosome-associated decay fragments are not present in either \textit{dcp2}Δ nor \textit{xrn1}Δ mutants (Fig. 3c and Fig. S6b, respectively). These data strongly indicate that decapping and 5\textsuperscript{-}3\textsuperscript{-} exonucleolytic digestion are essential for the generation of \textit{PGK1} \textit{RC} mRNA polyribosome-associated decay intermediates.

The \textit{PGK1} \textit{RC} mRNA polyribosome-associated decay fragments are not the result of No-Go\textsuperscript{14} decay since they persist in \textit{dom34}Δ mutants (Fig. S6c). The \textit{PGK1} \textit{RC} reporter is a minor substrate for No-Go decay, however, as demonstrated by No-Go decay fragments in \textit{xrn1}Δ cells\textsuperscript{14} (Fig. S6b; see *) and a slight stabilization of reporter half-life in \textit{dom34}Δ cells (Fig. S4). Importantly the No-Go decay fragments sediment on the top of the sucrose gradient, therefore, they are ribosome free (Fig. S6b).

\textbf{Ribosome recognition of the rare codon stretch is required for generation of decay intermediates associated with polyribosomes}

The experiments in Fig. 3b strongly argue that slowing translational elongation in \textit{cis} allows the detection of decapping on polyribosomes in wild-type cells. We repositioned the rare codon sequence within the reporter to further demonstrate that ribosome recognition is critical (Fig. S8a). Specifically, the same rare codon stretch was introduced to codons 390-400. Then we performed the polysome analysis followed by high resolution PAGE gel and Northern blot to detect the decay fragments using the 3\textsuperscript{'} end probe. Similar decay fragments were observed in the
polyribosome regions but importantly these were shifted in size appropriately (compare Fig. 3b to Fig. S8b). Specifically, fragments about 280-300nt were detected in the 80S, indicating they are associated with one ribosome, which is consistent with the distance from the rare codon stretch to the 3’ end of the reporter. In addition, decay fragments of increasing size were also observed in the heavier polyribosome regions, and these fragments’ length and the sedimentation position on the gradient correlate with the number of ribosome they can associate with. Collectively, these results indicate that the decay fragments we observed in the polyribosome regions require ribosome recognition of the rare codons.

**PGK1RC mRNA decay fragments are ribosome bound.**

To demonstrate an interaction between the RNA decay fragments and ribosomes, we purified ribosomes using an epitope-tagged 60S subunit\(^9\). The pelleted material was separated by sucrose gradient fractionation. We assayed for the localization of reporter RNA and observed that both full-length mRNA and the RNA decay fragments co-immunoprecipitated with epitope-tagged ribosomes (Fig. S9b). In contrast, decay fragments remained in the supernatant for untagged control cells (Fig. S9a). Ribosome immunoprecipitation has been shown to preferentially pellet monoribosomes and lighter polyribosomes\(^9\). The decay intermediates reflected this bias, i.e. smaller species co-immunoprecipitate with monoribosomes, and the larger fragments remain in the supernatant with the heavy polyribosomes. These data strongly argue that intermediates of mRNA degradation are associated with ribosomes and that mRNA decapping and exonucleolytic decay also occurs while the mRNA is bound by ribosomes.
Supplemental Figure Legends

Figure S1. A model of eukaryotic mRNA decapping. We propose that under normal circumstances mRNA decapping and exonucleolytic decay occur predominately while the mRNA is bound to polyribosomes. Deadenylation presumably reduces the rate of translational initiation and liberates the poly(A) binding protein. After deadenylation, decapping regulators can associate with the mRNA and presumably help facilitate loading of the decapping complex by displacing eIF-4F from the cap. Once the decapping complex is assembled, the mRNA is decapped at it’s 5’ end while still retained on polyribosomes. Exposure of the 5’ end monophosphate allows the exonuclease, Xrn1p, to degrade the mRNA co-translationally. Importantly, the direction of decay has evolved as to not impede the last translocating ribosome, thereby preventing the accumulation of truncated polypeptides.

Figure S2. mRNA decapping substrates sediment with polyribosomes

The poly(A) tail status of endogenous MFA2, PGK1, and RPL41A mRNA were analyzed by Northern blot in WT, dcp2Δ, and the deadenylase mutant ccr4Δ (a). The RNA in the dT lane was treated with RNaseH/oligo(dT) to indicate the deadenylated mRNA. (b) shows the quantification of the ratio of deadenylated mRNA to total mRNA in terms of percentage. Polysome profiles of WT and dcp2Δ cells are shown in (c). The relative positions of the non-translating region (RNP), 80S monoribosome peak, and polyribosomes peaks are indicated on the traces. (d) shows Northern blots of the endogenous PGK1 and RPL41A mRNA across the sucrose gradients in WT cells and dcp2Δ cells. The quantifications of mRNA in each fraction in terms of percentage of total are listed above the Northern blots. To detect the polyadenylation status of mRNA we have taken advantage of a novel RT-PCR approach developed by Pat Maroney, Sangpen.
Chamnongpol, and Tim Nilsen (in prep). (e) First, total RNA is treated with yeast Poly(A) Polymerase (yPAP) in the presence of high GTP and ITP. yPAP will add a poly(G) tail to the 3’ end of RNA under these conditions. Second, a poly(C) primer is added. Importantly, this poly(C) primer has two T residues on the 3’ end. Reverse transcription is performed followed by PCR using a message specific primer. (f) Shows a control reaction using the PAP tailing assay. Specifically the MFA2 mRNA was amplified from WT, dcp2Δ (decapping mutant), and ccr4Δ (deadenylase mutant) cells. -PAP: indicates sample in which yPAP was omitted. -RT: indicates sample in which reverse transcriptase was omitted. A0 indicates size of completely deadenylated MFA2 mRNA. Size markers are indicated on the outside lanes (in nucleotides). PCR products were resolved on a 3% agarose gel and visualized with SYBRGold (Invitrogen). (g) shows the poly(A) tail status of the MFA2 mRNA across a sucrose gradient in WT cells or dcp2Δ cells (h). Fractions are indicated above each lane, as are the relative positions of the non-translating region (RNP), 80S monoribosome peak, and polyribosomes peaks. A0 indicates the migration of the fully deadenylated MFA2 mRNA. Size markers are indicated to the left of each gel in nucleotides. The ORF size for MFA2 mRNA is 39 codons.

**Figure S3. Endogenous decapped mRNAs are ribosome bound.** Polyribosome affinity purification was performed in xrn1Δ cells (a) and xrn1Δ cells with Rpl25 FLAG tagged (b). The traces of the purified polyribosomes and the supernatants are shown in (a) and (b). Primer extension for endogenous CYH2 and ADH1 mRNAs were performed in the purified polyribosomes from xrn1Δ cells and xrn1Δ cells with Rpl25 FLAG tagged (c), and the U1 snRNA was used as a control (c).
Figure S4. PGK1RC mRNA decays via decapping-dependent, 5’-3’ decay. Transcription shut-off analysis was used to analyze the half-lives of PGK1RC reporter in WT and different mRNA decay mutant cells. Time points are indicated above the panel in minutes. Half-lives shown were calculated after normalization for the amount of 7S RNA in each lane.

Figure S5. Size heterogeneity of the decay fragments results from the 5’ end of the PGK1RC reporter. Cell lysate from WT cells expressing the PGK1RC reporter was passed through the sucrose density gradient, and RNAs were extracted from each fractions of the gradient, and then analyzed by polyacrylamide Northern blots using the different probes depicted in the upper panel. The relative positions of the non-translating region (RNP), 80S monoribosome region, polyribosome regions are indicated above the fraction numbers.

Figure S6. The mRNA decay fragments in the polyribosome regions are decapping and 5’-3’ exonucleolytic digestion products. Polyribosome analyses were performed on WT cells (a), xrn1Δ cells (b), and dom34Δ cells (c) expressing the PGK1RC reporter. The RNAs from fractions across the sucrose gradients were extracted, and then subject to polyacrylamide Northern blot analysis using the 3’ end probe depicted in Fig. 3b. The relative positions of the non-translating region (RNP), 40S ribosome subunit, 60S ribosome subunit, 80S monoribosome region, and polyribosome regions are indicated on the traces. * indicates the No-Go decay fragments in xrn1Δ cells.

Figure S7. Formation of the decay fragments on polyribosome regions requires mRNA translation. The SL-PGK1RC, PGK1RC and PGK1PTC-RC reporters are depicted in (a). WT cells
expressing SL-\textit{PGK1}^\text{RC} reporter (b), \textit{upf1}Δ cells expressing \textit{PGK1}^\text{RC} reporter (c) and \textit{PGK1}^{PTC-RC} reporter (d) were subject to polyribosome analysis, followed by Northern blots using the pG probe.

**Figure S8. Ribosome recognition of the rare-codons are required for generation of decay intermediates.** (a) depicts a \textit{PGK1} mRNA reporter with the same rare codon stretch as Fig. 3A inserted at a different region of the coding region. (b) shows the polysome analysis of WT cell with the new rare codon reporter followed by high resolution PAGE gel and Northern blot using the probe depicted in (a) to detect the decay fragments on the sucrose gradient.

**Figure S9. \textit{PGK1}^\text{RC} mRNA decay fragments are ribosome bound.** Polyribosome affinity purification was performed in both the WT cells (a) and Rpl25p FLAG-tagged cells (b) expressing the \textit{PGK1}^\text{RC} reporter. The purified polyribosome and the supernatant were subject to sucrose density gradient analysis followed by Northern blot using the 3’ end probe of the \textit{PGK1}^\text{RC} reporter.

**Figure S10. The primer extension analysis for - cap mRNA is quantitative.** Primer extension analysis was performed on different amounts of total RNA from \textit{xrn1}Δ cells (a). The amount of RNA used spans the range seen across sucrose gradients in Fig. 1 & 2. The full length (FL) and decapped (- cap) mRNA are indicated on the panel. Quantification of FL and - cap mRNAs is presented in (b), with calculated R and P values. The ratio of FL to - cap mRNAs over different amounts of total RNA is shown in (c).
Hu et al., Supplemental Figure 1

Deadenylation

Polysome-associated decapping

Co-translational 5'→3' Exonucleolytic decay
Hu et al., Supplemental Figure 2

(a) Gel images of RNA samples from different conditions:
- MFA2 mRNA
- PGK1 mRNA
- RPL41A mRNA

(b) Histogram showing the ratio of deanylated to total RNA for MFA2, PGK1, and RPL41A mRNA under WT, dcp2Δ, and ccr4Δ conditions.

(c) Electrophoretic analysis of RNP and polyribosome peaks for WT and dcp2Δ conditions.

(d) Bar charts and gel images showing the percentage of RNP, 80S, and polyribosomes for WT and dcp2Δ conditions.

(e) Diagram illustrating the yPAP GTP:ITP RT PCR processes.

(f) Gel analysis of different mRNA conditions (A0, WT, dcp2Δ, ccr4Δ), showing amplified fragments.

(g) Comparison of MFA2 mRNA levels under WT and dcp2Δ conditions.

(h) Comparison of MFA2 mRNA levels under WT and dcp2Δ conditions.
Hu et al., Supplemental Figure 3

(a) Untagged

(b) FLAG-Rpl25p

(c) Total RNA | Pellet

| Untagged | Untagged | Flag-RPL25 | Flag-RPL25 | Untagged | Flag-RPL25 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| WT | xrn1Δ | WT | xrn1Δ | WT | xrn1Δ |

- CYH2
- ADH1
- U1 snRNA

- FL - cap
### Table: Half-life (min.)

| Strain | Half-life (min.) |
|--------|-----------------|
| WT     | 9.5             |
| ccr4Δ  | 56.2            |
| dhhlΔ  | 27.9            |
| km1Δ   | 23.6            |
| dcp2Δ  | 53.2            |
| xrn1Δ  | 65.6            |
| upflΔ  | 9.7             |
| ski7Δ  | 9.9             |
| dom34Δ | 12.3            |
| HCHO   | > 60            |

### Graph: Half-life (min.)

Graph showing the half-life of different strains compared to WT.
Hu et al., Supplemental Figure 5
Hu et al., Supplemental Figure 6

(a) RNP 60S 40S 80S
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

(b) RNP
60S 40S 80S
Polyribosomes
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

(c) RNP
80S
Polyribosomes
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

WT
xrn1Δ
dom34Δ
a

SL-PGK<sub>RC</sub>

PGK<sub>RC</sub>

PGK<sub>PTC-RC</sub>

UAG Stop
(Codon 302)

b
c
d

Reporter: SL-PGK<sub>RC</sub>  PGK<sub>RC</sub>  PGK<sub>PTC-RC</sub>

Strain: WT  upf1Δ  upf1Δ
Codon 390-400
Leu  Ile  Ala  Arg  Arg  Arg  Arg  Ala  Thr
TTAATA GCG CGG CGG CGG CGG CGG GCG ACG

7GpppN ---------------------------------------- 416 amino acids ---------------------------------------- AAAAn

3 Probe

1562 nt
287 nt
180 nt

mRNP

Polyribosomes

40S
60S
80S

1 2 3 4 5 6 7 8 9 10 1112 131415

501nt
489nt
404nt
353nt
243nt
Table 1. Yeast Strains

| Strain Name | Genotype | Source |
|-------------|----------|--------|
| yJC151      | MATa, ura3, leu2, his3, met15 | EUROSCARF |
| yJC162      | MATa, ura3, leu2, his3, met15, ccr4::NEO | EUROSCARF |
| yJC167      | MATa, ura3, leu2, his3, met15, lsm1::NEO | EUROSCARF |
| yJC173      | MATa, ura3, leu2, his3, met15, ski7::NEO | EUROSCARF |
| yJC182      | MATa, ura3, leu2, his3, met15, xrn1::NEO | EUROSCARF |
| yJC287      | CB012: MATa, ade2-1, his3, leu2, trp1, ura3, pep4::HIS3, prb::HIS3, pre1::HIS3 | Inada et al, 2002 |
| yJC288      | YIT613: MATa, ade2-1, his3, leu2, trp1, ura3, pep4::HIS3, prb::HIS3, pre1::HIS3, rpl25::LEU2 [pRPL25-Flag-URA3-CEN] | Inada et al, 2002 |
| yJC327      | MATa, ura3, leu2, his3, met15, dcp2::NEO | Ambro van Hoof |
| yJC330      | MATa, ura3, leu2, his3, met15, dhhl::NEO | EUROSCARF |
| yJC443      | MATa, ura3, leu2, his3, met15, upf1::NEO | EUROSCARF |
| yJC444      | MATa, ura3, leu2, his3, met15, dom34::NEO | EUROSCARF |
| yJC463      | CB012: MATa, ade2-1, his3, leu2, trp1, ura3, pep4::HIS3, prb::HIS3, pre1::HIS3, xrn1::NEO | This study |
| yJC464      | YIT613: MATa, ade2-1, his3, leu2, trp1, ura3, pep4::HIS3, prb::HIS3, pre1::HIS3, rpl25::LEU2, xrn1::NEO, [pRPL25-Flag-URA3-CEN] | This study |
| yJC489      | MATa, ura3, leu2, his3, met15, xrn1::NEO, upf1::LEU2 | This study |
| Name       | Description                                                                 | Reference                  |
|------------|------------------------------------------------------------------------------|----------------------------|
| pJC134     | pRP543 (SL-PGK1)                                                            | Coller and Parker, 2005    |
| pJC296     | PGK1pG reporter under control of GAL1 promoter                              | Decker and Parker, 1993    |
| pJC313     | MFA2 riboprobe                                                              | Muhlrad et al, 1994        |
| pJC331     | PGK1pG with a unique tag for primer extension                               | Muhlrad et al, 1994        |
| pJC314     | PGK1pG reporter with a rare codon stretch                                   | This study                 |
| pJC320     | PGK1pG-RC reporter with a stem loop in 5'UTR                               | This study                 |
| pJC327     | PGK1pG-RC reporter with a PTC at codon 302                                 | This study                 |
| pJC325     | MFA2pG reporter with a rare codon stretch                                   | This study                 |
| pJC349     | PGK1-Short Construct                                                        | This study                 |
| pJC372     | PGK1pG reporter with a rare codon stretch at 390-400                        | This study                 |
| oRP100     | 5'-GTCTAGCCGCGAGGAAGG-3'                                                    | Muhlrad et al., 1995       |
| oRP121     | 5'-AATCCCCCCCCCCCCCCCCCCCCA-3'                                              | Muhlrad et al., 1995       |
| oJC124     | 5'-TTAGAGTTTATTTACTCATATAATCCGC-3'                                         | This study                 |
| oJC305     | 5'-TTAGCGTAAAGAGATGGGG-3'                                                   | This study                 |
| oJC131     | 5'-GAATGCTAATTTATGAAAGAC-3'                                                 | This study                 |
| oJC556     | 5'-CGTCGGCCGCGCGCGCGCGCTTTTAGGATCCATTTGACCATTTGTCAACC-3'                   | This study                 |
| oJC557     | 5'-TTAGCTAGGCGGCGGCGCGCGGCGGACGACACATTGCTCTGAAAGCTTACCAACCAG-3'             | This study                 |
| oJC558     | 5'-CCGGGGGATCCGTACTTTACTCTTCTCT-3'                                         | This study                 |
| oJC559     | 5'-GTGCGCAACCTTTACAGAACAGCAAT-3'                                           | This study                 |
| oJC586     | 5'-CGTCGGCCGCGCGCGCGCGGCGCTTTGAA-3'                                        | This study                 |
| oJC588     | 5'-TCAGCGAATCTTTGCTACCACTTTACCAAC-3'                                       | This study                 |
| oJC590     | 5'-TCTGCTCTATTAGGATCTCATTACCAAC-3'                                         | This study                 |
| oJC591     | 5'-CGGATAGAAAAGACCCGACACTTGGAC-3'                                          | This study                 |
| oJC611     | 5'-AACACCAAGACTGTCGACTGACAGGATTTCCAGCTGCTGAG-3'                            | This study                 |
| oJC612     | 5'-CCACCGAGTTGGAATACTCCCTTACTCTCTCAGTCTGCTGCTGTTT-3'                       | This study                 |
| oJC616     | 5'-TATGCTCTGTCTTTACATCTCTCGTGAG-3'                                         | This study                 |
| oJC620     | 5'-GATCAGAATTTCTGCTGCGTCTAATAAGAAGACAA-3'                                  | Muhlrad et al., 1995       |
| oJC635     | 5'-CTGCTCTCTCTCTTACCTCGACG-3'                                               | This study                 |
| oJC638     | 5'-TACGACCTTTATCCGGTGAGACGTGACTCTC-3'                                       | This study                 |
| oJC639     | 5'-GGTAAATAGCAGCTACTATAGGAGACCACCCCCCCCCCCCCCCCTATAGGAGACGTGACTCTC-3'      | This study                 |
| oJC652     | 5'-ACCAAGGAGTTTGTCACTTGAATGAC-3'                                           | This study                 |
| oJC706     | 5'-GCCUGAAGGGGAGGAAGAAGAACACGCCGUCUGUGCUUUGAAGAAA-3'                       | This study                 |
| oJC707     | 5'-GCTGCGAATCCGTCGACTGACAGGATGCAA-ACTGAC-3'                                | This study                 |
| oJC789     | 5'-CATGAAAAATCTGGTAAAGTGAATACACTAC-3'                                       | This study                 |
| oJC790     | 5'-GAGGTGGAATCTTACCAATCTTACCC-3'                                           | This study                 |
| oJC791     | 5'-CTAATTTATTTCAATATTTCATCTCCATTAGC-3'                                     | This study                 |
| oJC824     | 5'-TTAGCTACTTTATCATTACCAATTTGACGTTGCTTACCC-3'                              | This study                 |
| oJC825     | 5'-GCTTCGCCGCGCCTCGCCGCGGCGGTATAGTAAACATGGGAGATCTTTATGCTGCTGACG-3'          | This study                 |
| oJC826     | 5'-ATTCGTTTTTATATTTTTGCTTTAAAGATGATAATTTACTTCTCTT                          | This study                 |
| oJC837     | 5'-GGTTCGAGTCACACTCTGAGTTGAGAGCTGACG-3'                                   | This study                 |
| oJC838     | 5'-GGTTCGACCTCGAATGAGGTTGCTCATTCCAAGACGCAAACGCCATGGTGCTT-3'                | This study                 |
| oJC839     | 5'-CTGCGCTCTCACTTCCGCTTCCTTTCTCTTA-3'                                     | This study                 |