mRNA 3' tagging is induced by nonsense mediated decay and promotes ribosome dissociation.

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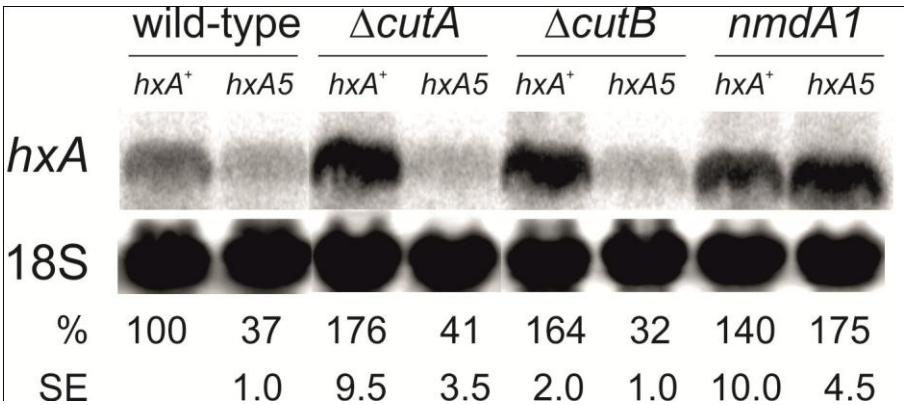
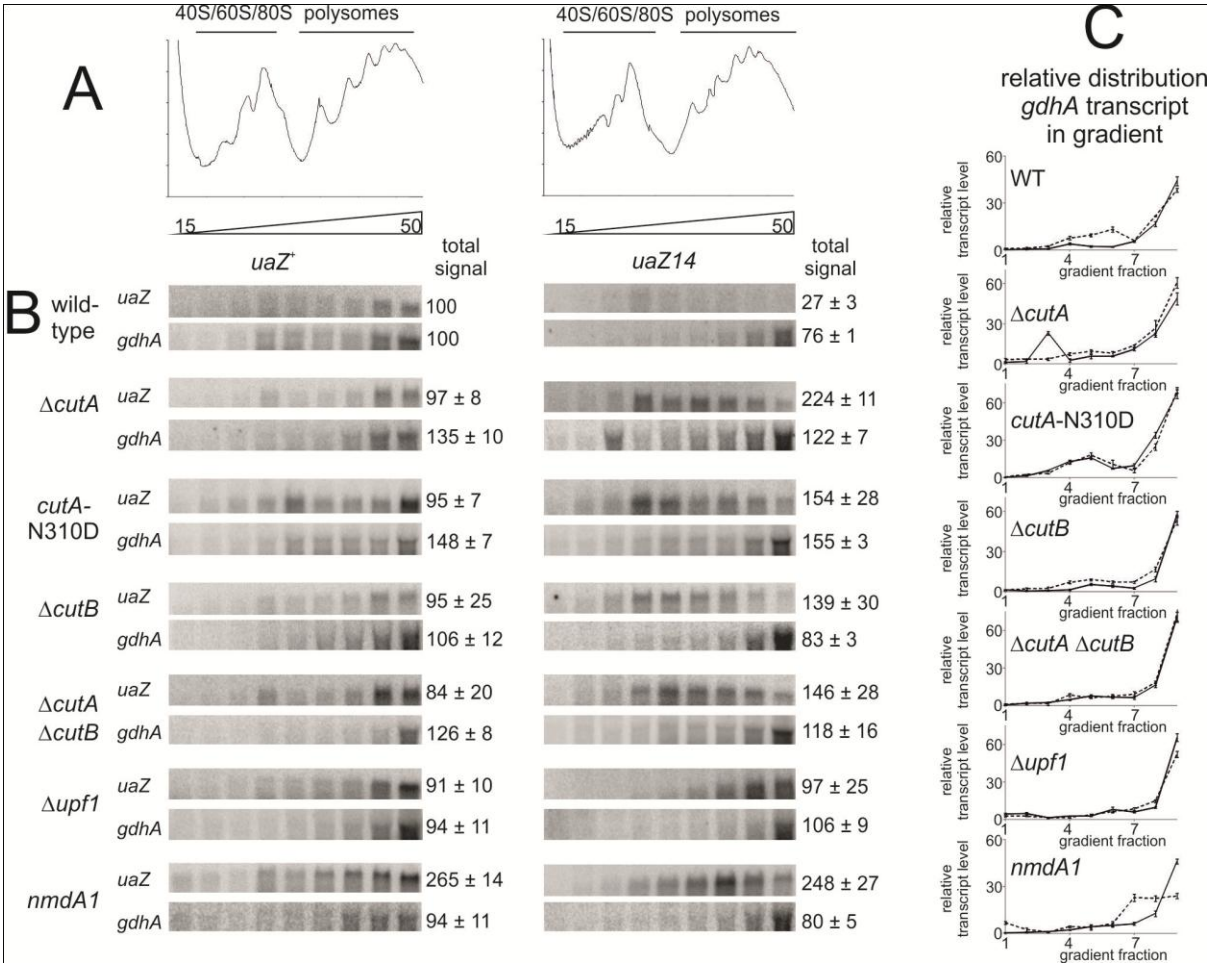
Supplementary Figures S1-S4



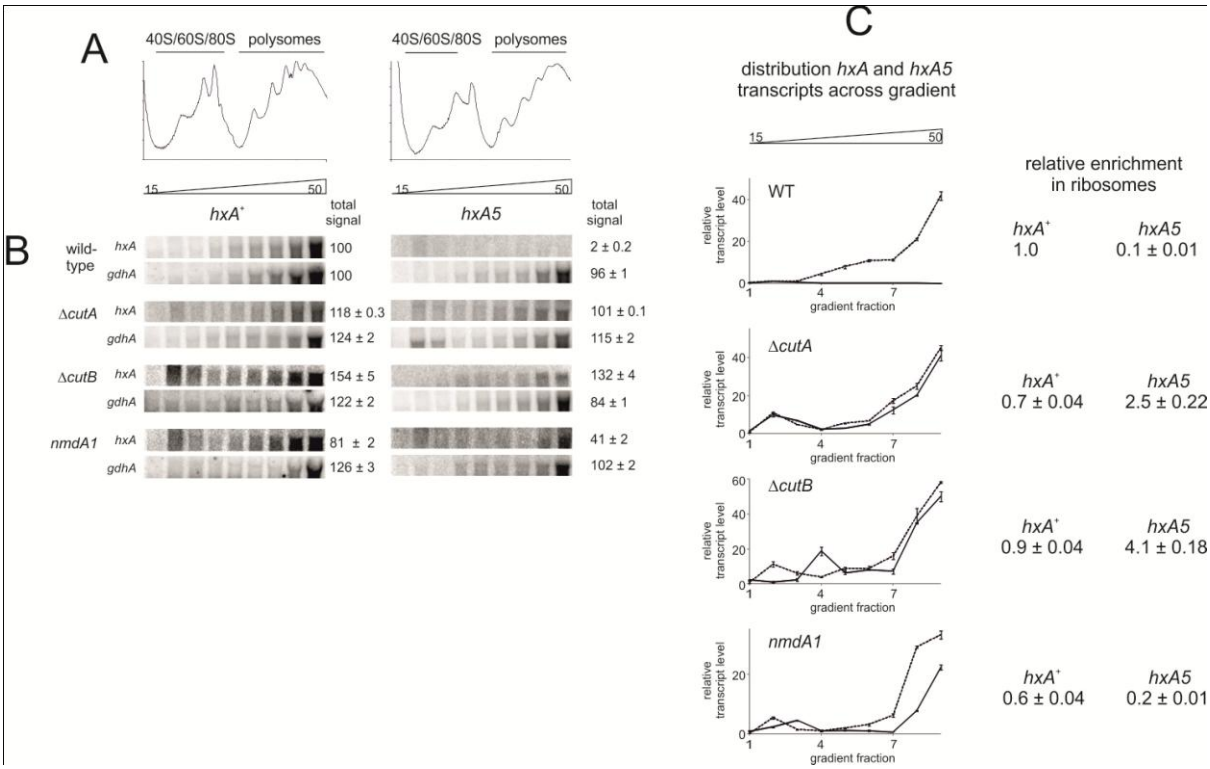
Supplementary Figure S1. Disruption of *cutA* or *cutB* does not suppress NMD of *hxA5* mRNA. Northern blot analysis was conducted to monitor the level of expression of the *hxA*$^{+}$ and *hxA5* transcripts in wild-type, Δ*cutA*, Δ*cutB*, and *nmdA1* strains. The strains were grown overnight for 16 hours in the presence of sodium nitrate as the nitrogen source. The overnight cultures were washed and transferred to media containing uric acid (0.1 mg ml^{-1}) for 2 hours in order to induce expression of *hxA*. 18S rRNA was used as a loading control. Multiple northern blots (2) were quantified and the average level of *hxA* expression is given (%). SE, standard error is shown.



Supplementary Figure S2. Polysome fractionation analysis of *gdhA* and *uaZ* mRNA

(**A**) Representative UV absorbance profiles of cellular extracts from different strains after velocity sedimentation through a 15-50% sucrose gradient. The positions of 40S and 60S ribosomal subunits as well as the mono- (80S) and polyribosomes are shown above the UV profiles. Each experiment was repeated at least three times and representative data are shown. (**B**) Distribution of polyribosome associated *uaZ*$^{+}$, *uaZ14* and *gdhA*$^{+}$ mRNAs from different strains (wild-type, Δ*cutA*, *cutA*-N310D, Δ*cutB*, Δ*cutA* Δ*cutB,* Δ*upf1* and *nmdA1*) as northern blot signals. "Total signal" shown at the right of each northern indicates the average signal (including standard error of at least three independent experiments) of the

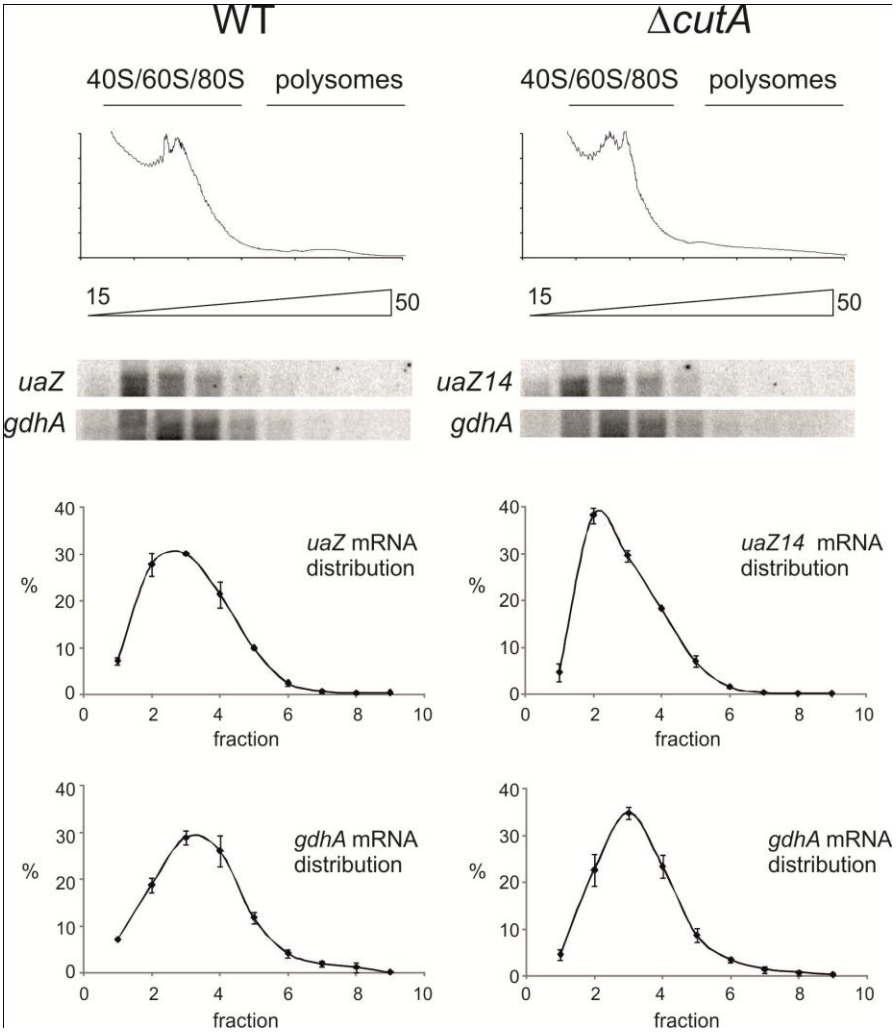
corresponding mRNA in whole sucrose gradients which were assayed simultaneously, relative to the wild type signal as 100%. (**C**) Distribution of polyribosome associated *gdhA*$^{+}$ transcript in the strains. A 10 ml gradient was separated into nine equal fractions and RNA was extracted from each fraction and subjected to northern analysis. Dotted lines represent the *gdhA*$^{+}$ transcript in *uaZ*$^{+}$ strains, solid lines in *uaZ14* strains. Error bars show standard errors. The data points represent the relative intensity of each fraction relative to the total combined intensity of all fractions for each gradient. This is scaled relative to *gdhA*$^{+}$ in the wild type to facilitate comparisons.



Supplementary Figure S3. CutA and CutB are implicated in ribosome dissociation from *hxA5* mRNA.

Polyribosome fractionation was conducted for *hxA*$^{+}$ (encoding xanthine dehydrogenase) and *hxA5* (where a transition C1709T results in an amber mutation that truncates the protein prematurely after residue 369) strains. The genetic background for each strain is indicated (wild-type, Δ*cutA*, Δ*cutB*, and *nmdA1*). (**A**) Representative UV absorbance profiles of cellular extracts separated by velocity sedimentation through a 15-50% sucrose gradient are given. Each 10 ml gradient was separated into 9 equal fractions and the RNA extracted from each subjected to northern analysis for *hxA* and *gdhA* transcripts. The position of 40S and 60S ribosomal subunits as well as mono- (80S) and polyribosomes are shown above the UV

profiles. Each experiment was repeated at least three times and representative data are shown. (**B**) Distribution of polyribosome associated *hxA*$^{+}$, *hxA5* and *gdhA*$^{+}$ mRNAs from different strains (wild-type, Δ*cutA*, Δ*cutB* and *nmdA1*). "Total signal" shown at the right of each northern indicates the average signal (including standard error of two independent experiments) of the corresponding mRNA in whole sucrose gradients which were assayed simultaneously, relative to the wild type signal as 100%. (**C**) Distribution of polyribosome associated *hxA*$^{+}$ and *hxA5* mRNAs from these strains. A 10 ml gradient was separated into nine equal fractions and RNA was extracted from each fraction and subjected to northern analysis. Dotted line is the *hxA*$^{+}$ mRNA, solid lines represents the *hxA5* transcript. Mean values with standard errors are shown from two replicate experiments. The data points represent the relative intensity of each fraction relative to the total combined intensity of all fractions for each gradient. This is scaled relative to *hxA*$^{+}$ in the wild type to facilitate comparisons. (The low signal from the *hxA* transcript in the *hxA5* strain is primarily in fractions 2 and 3). The relative proportions of *hxA*$^{+}$ and *hxA5* mRNAs in ribosome association fractions versus total mRNA are given on the right-hand side. The northern data for total mRNA (summarised in Fig. S1) were compared to the combined signal from the pooled fractionated (ribosome associated) samples as determined by quantitative northern analysis.



Supplementary Figure S4. Polyribosome profile and mRNA analysis in the presence of EDTA.

To examine whether mRNA was associated with the ribosomes during sucrose gradient centrifugation, rather than sedimenting as non-ribosomal RNA-protein complexes, the cytoplasmic extracts from WT and Δ*cutA uaZ14* strains were treated with 10 mM EDTA. This treatment leads to the dissociation of ribosomes from mRNA without disrupting non-ribosomal RNA-protein complexes (1). Cells were harvested and cytoplasmic extracts prepared as described in Material and Methods except that 10 mM EDTA was included in the wash, extraction and sucrose gradient buffers. The data points represent, as percentages, the total amount of *uaZ*$^{+}$, *uaZ14* and *gdhA*$^{+}$ mRNAs recovered over each

gradient, with standard errors. EDTA treatment resulted in the disruption of polyribosomes and loss of the mRNA normally associated with these fractions (compare with Figs. 5, S2 and S3). This indicates that the presence of mRNAs in the polyribosomal fractions in the absence of EDTA is due to its association with ribosomes and not with non-ribosomal RNA-protein complexes. Puromycin treatment, 30 μg ml^{-1} (2) was also shown to partially disrupt polysome fractions. However, in the absence of cycloheximide, which is used to freeze the interaction between the ribosomes and mRNA, no signal was detected for either *uaZ*$^{+}$ or *gdhA*$^{+}$.

References:

1. **Calzone, F. J., R. C. Angerer, and M. A. Gorovsky.** 1982. Regulation of protein synthesis in Tetrahymena: isolation and characterization of polysomes by gel filtration and precipitation at pH 5.3. Nucleic Acids Res **10:**2145-2161.
2. **You, K. T., L. S. Li, N. G. Kim, H. J. Kang, K. H. Koh, Y. J. Chwae, K. M. Kim, Y. K. Kim, S. M. Park, S. K. Jang, and H. Kim.** 2007. Selective translational repression of truncated proteins from frameshift mutation-derived mRNAs in tumors. PLoS Biol **5:**e109.