Polysome Fractionation Analysis Reveals Targets of Nonsense-Mediated mRNA Decay are Preferentially in the Monosome Fraction and Supports the 50nt Rule as a Predictor of NMD

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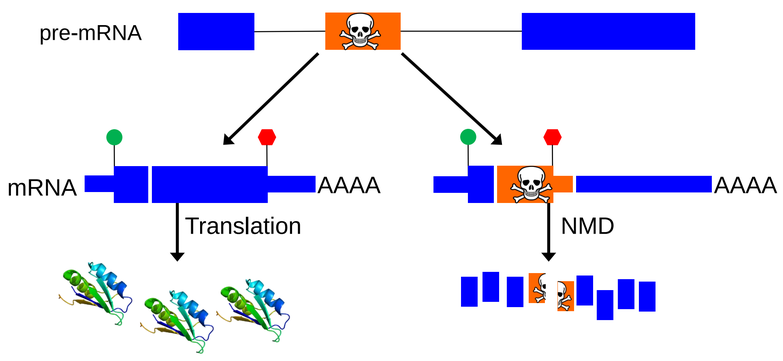
The nonsense-mediated mRNA decay pathway regulates gene expression

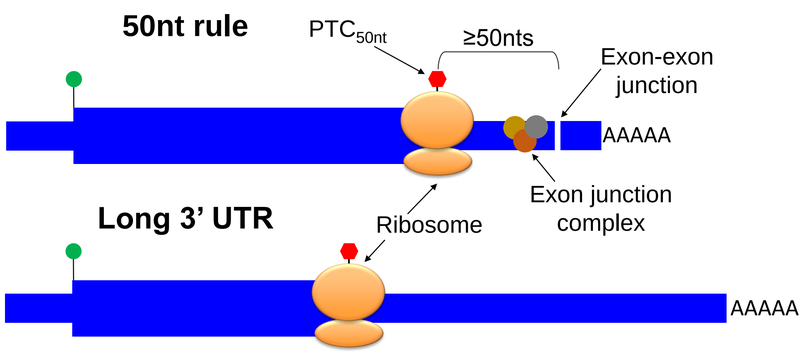
The nonsense-mediated mRNA decay (NMD) pathway regulates thousands of genes in human cells, many of which are known to be regulated during stress responses and development. NMD degrades transcripts with a premature termination codon (PTC) but what defines a stop codon as premature, rather than normal, is poorly understood. Here we use RNA-seq data from a polysome fraction experiment to better characterize NMD targeting features in human cells.

Alternative splicing can introduce a PTC into a transcript



What targets transcripts to NMD?

In mammals, the canonical model is that a **PTC$_{50nt}$** targets a transcript to NMD. A PTC$_{50nt}$ is a stop codon located 50 nucleotides or more upstream of an exon-exon junction [1]. There is also evidence that a long 3' UTR can trigger NMD in yeast, plants, flies and mammals [2]. NMD targets are expected to be degraded during the pioneer round of translation [3].

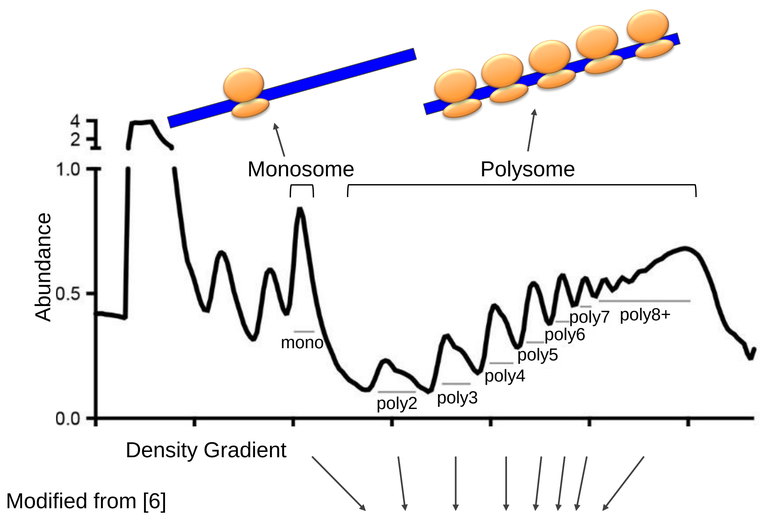


Given that NMD targets are expected to be degraded during the pioneer round of translation, we predict that many NMD targets would be bound by one or only a few ribosomes. NMD targets in yeast are predominantly found in the monosome fraction [4] and in humans, exons with stop codons in all three reading frames are depleted from the polysome fraction [5]. Therefore, we want to:

1. Investigate whether NMD targets are predominantly found in the monosome fraction in human cells
2. Find NMD-associated features that target transcripts to the monosome fraction in order to support their link to NMD targeting

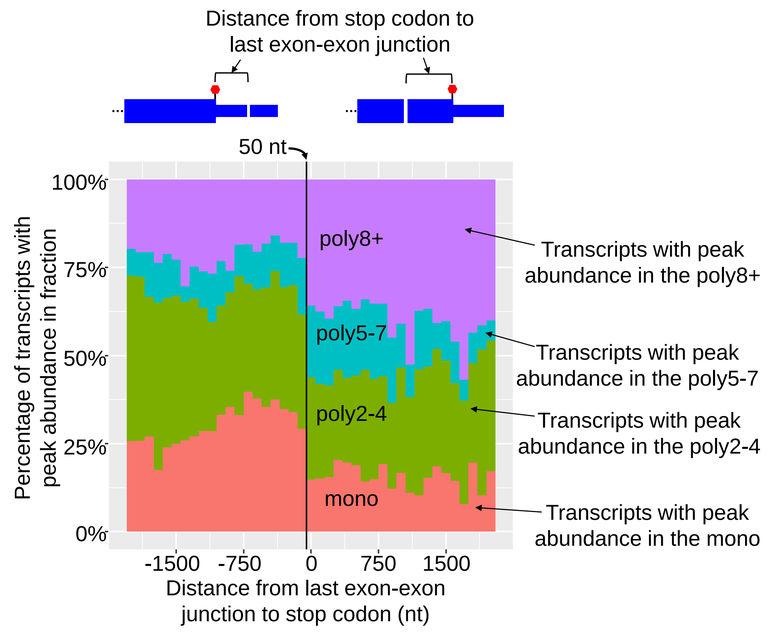
RNA-seq of polysome fractions reveals the translation state of transcripts

Transcripts bound by ribosomes can be separated on a density gradient. These transcripts can be sequenced after ribosome depletion and cDNA synthesis. Using such data we can determine if NMD targets are abundant in the monosome fraction and use monosome fraction abundance to inform our understanding of the mechanisms of NMD targeting. We re-analyzed polysome fraction data from Floor and Doudna [6] to answer these questions.



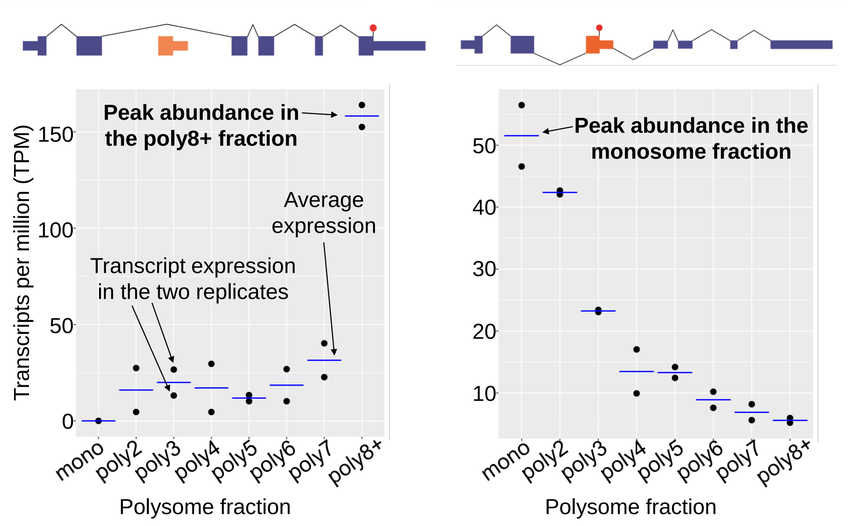
Transcripts with a PTC$_{50nt}$ are enriched in the monosome fraction

Transcripts with an PTC$_{50nt}$ are more likely to have peak expression in the monosome fraction than transcripts without. This is consistent with 3' UTR exon-exon junctions and the exon junction complex being potent stimulators of NMD.



SRSF6: Productive isoform

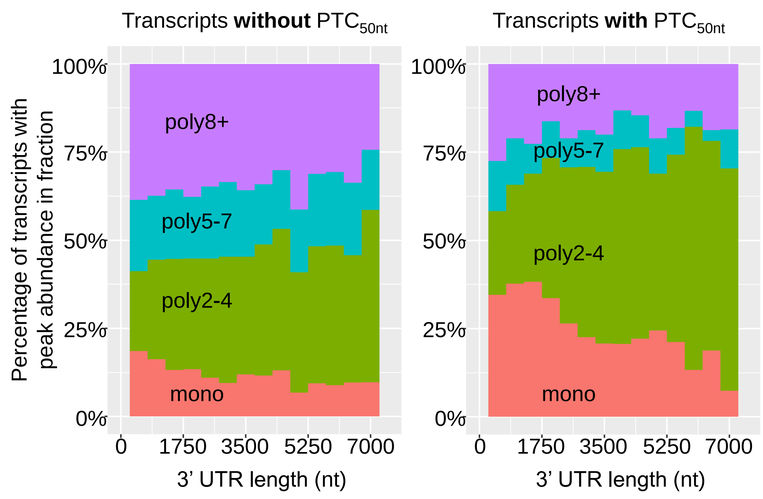
SRSF6: NMD targeted isoform



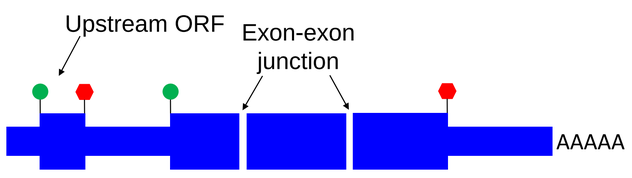
As an example, SRSF6 has a protein productive isoform and an NMD targeted isoform. The protein productive isoform is highly abundant in the poly8+ fraction, consistent with it being highly translated. The NMD targeted isoform has peak abundance in the monosome fraction, consistent with being targeted to NMD.

Transcripts with longer 3' UTRs are not enriched in the monosome fraction

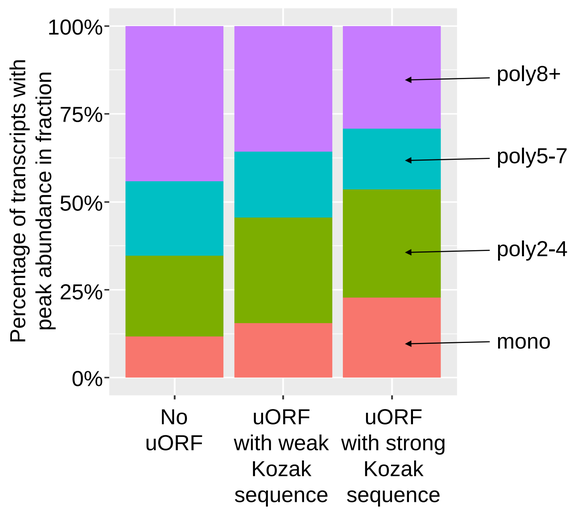
Transcripts with long 3' UTRs are not enriched in the monosome fraction compared to other transcripts, suggesting that long 3' UTRs are not a major feature targeting transcripts to NMD. However, long 3' UTR transcripts could be targeted to NMD after the pioneer round of translation.



Upstream ORF-containing transcripts are enriched in the monosome fraction



Upstream ORFs (uORFs) are short open reading frames located in the 5' leader sequence. Some are known to be translated and target transcripts to NMD. We find that more uORF-containing transcripts are abundant in the monosome fraction than transcripts without an uORF and a strong Kozak sequence around the uORF start codon leads to more monosomal transcripts.



Conclusions

- Transcripts with a PTC$_{50nt}$ are more abundant in the monosome fraction than other transcripts
- Longer 3' UTR transcripts are not enriched in the monosome fraction
- Upstream ORF-containing transcripts are more abundant in the monosome fraction, indicating that some uORFs target transcripts to NMD
- The use of polysome fraction data could be used to distinguish between direct targets of NMD and the many indirect targets that respond to NMD inhibition

Acknowledgements

We acknowledge the Center for RNA Systems Biology at UC Berkeley (NIH P50 GM102706 Jamie Cate) for funding James Lloyd, NDSEG Fellowship for funding Courtney French, the Tang Distinguished Scholarship to Gang Wei, and NIH R01-GM0716555 to Steven Brenner. We thank Stephen Floor for advice on analysis of the polysome data.

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