Illuminating nonsense-mediated mRNA decay by polysome fractionation

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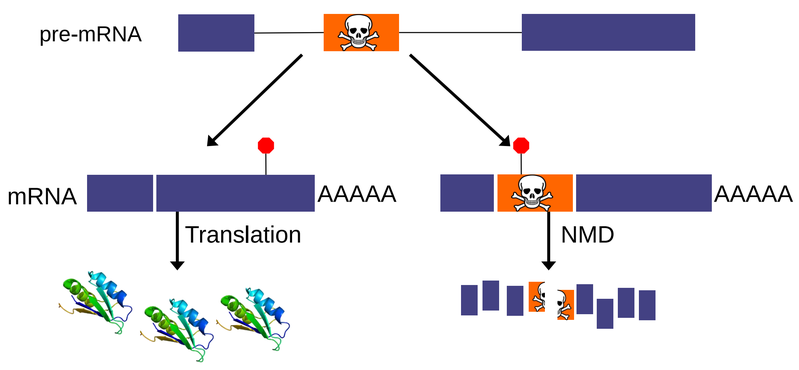
Alternative splicing coupled to nonsense-mediated mRNA decay regulates gene expression

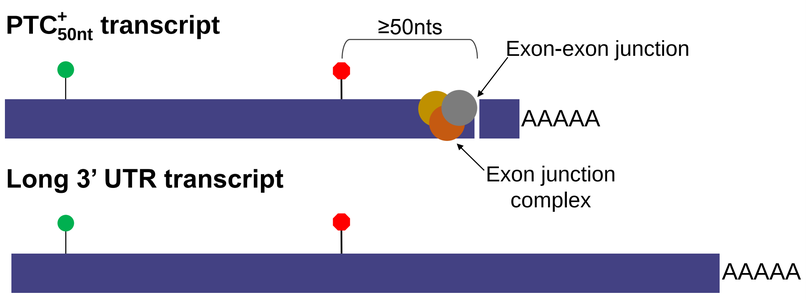
Alternative splicing coupled to nonsense-mediated mRNA decay (NMD) pathway regulates thousands of genes in human cells. NMD degrades transcripts with a premature termination codon (PTC) although the features that define a stop codon as premature are poorly understood. Here we use RNA-seq data from a polysome fraction experiment to better characterize NMD targeting features in human cells.

Alternative splicing can differentially introduce a PTC



What targets transcripts to NMD?

In mammals, exon-junction complex (EJC) model states that a **PTC$_{50nt}$** targets a transcript to NMD. A PTC$_{50nt}$ is a stop codon located ≥50 nucleotides of an exon-exon junction [1]. But there is also evidence that a long 3' UTR can trigger NMD in yeast, plants, flies and mammals [2].



Hypothesis

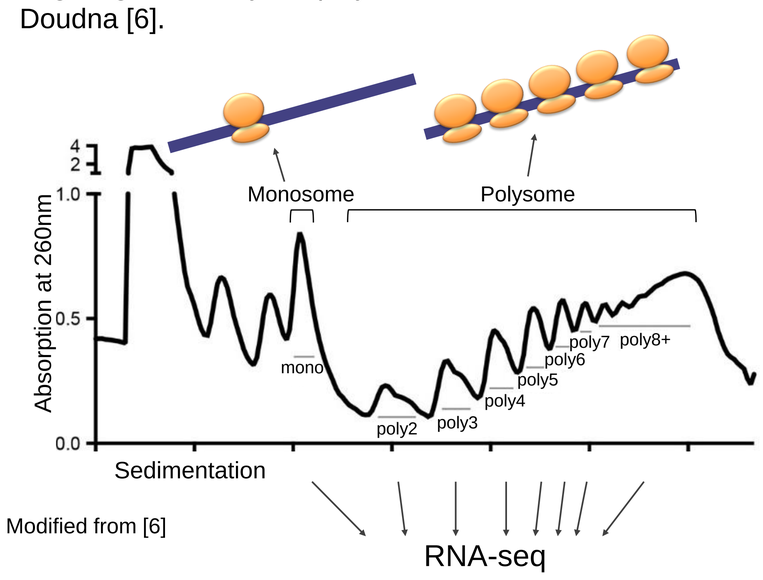
Given that NMD targets are expected to be degraded during the pioneer round of translation [3], we predict that many would be bound by a single ribosome. NMD targets in yeast are predominantly found in the monosome fraction [4] and in humans, exons harboring PTCs are depleted from the polysome fraction [5].

Aims

1. Investigate whether NMD targets are predominantly found in the monosome fraction
2. Test if features proposed to trigger NMD lead to an accumulation of transcripts in the monosome fraction

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

Transcripts bound by ribosomes can be separated into fractions on a mass gradient and then sequenced. 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 analyzed polysome fraction data from Floor and Doudna [6].

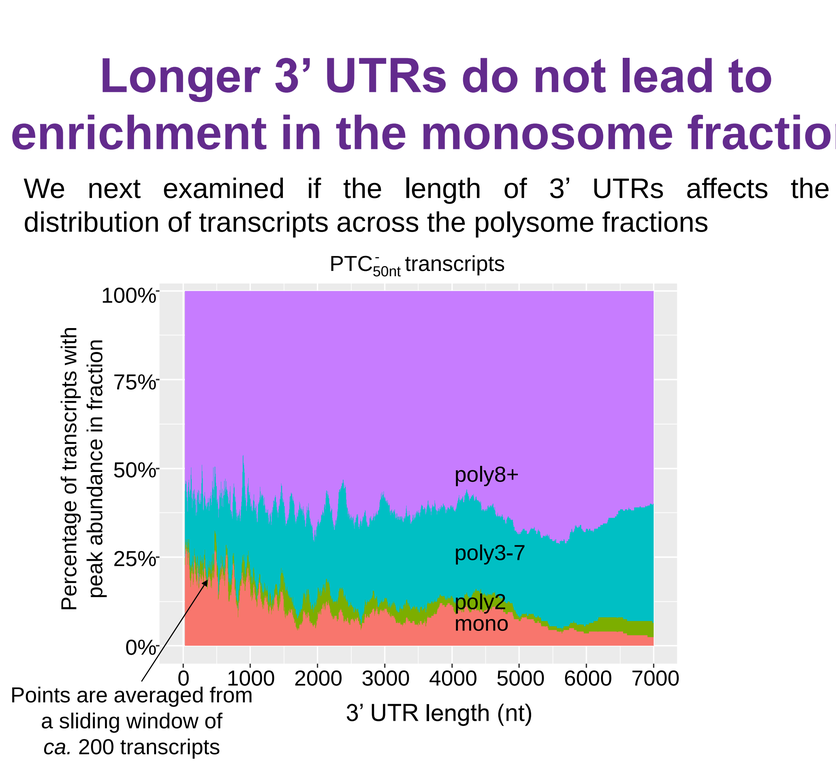


Modified from [6]

We normalized by taking the transcript abundance (transcripts per million; TPM) and adjusted for the amount of RNA in each fraction (area under the curve), while accounting for the ratio of mRNA to rRNA in each fraction.

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

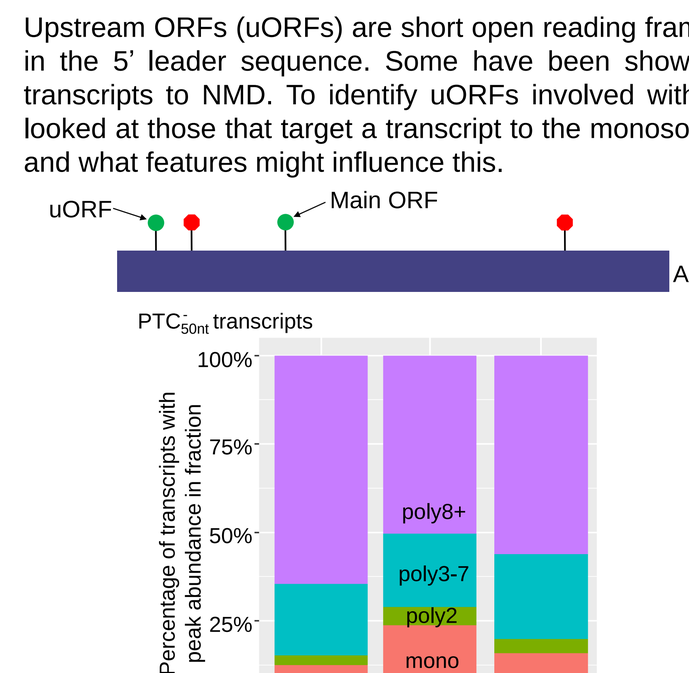
Below is a example where the inclusion of a single exon with a PTC$_{50nt}$ that changes the accumulation pattern across the polysome fractions.



- The PTC$^{-}_{50nt}$ transcript isoform is highly abundant in the poly8+ fraction, consistent with it being highly translated
- The PTC$^{+}_{50nt}$ targeted isoform has peak abundance in the monosome fraction, consistent with being targeted to NMD

Longer 3' UTRs do not lead to enrichment in the monosome fraction

We next examined if the length of 3' UTRs affects the distribution of transcripts across the polysome fractions



- Transcripts with long 3' UTRs are not enriched in the monosome fraction compared to other transcripts
- This suggests 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 have been shown to target transcripts to NMD. To identify uORFs involved with NMD, we looked at those that target a transcript to the monosome fraction and what features might influence this.

- uORF-containing transcripts are more abundant in the monosome fraction than transcripts without an uORF
- A strong Kozak sequence around the uORF start codon leads to more monosomal transcripts

Conclusions

- Many PTC$^{+}_{50nt}$ transcripts accumulate in the monosome fraction. This is consistent with these transcripts being NMD targets
- Longer 3' UTR transcripts are not enriched in the monosome fraction. This suggests that they are not targeted to NMD, at least during the pioneer round of translation
- Upstream ORFs with strong start codon contexts appear to be potent triggers of NMD and/or translation repression of transcripts

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References

1. Nagy E and Maquat LE. (1998) A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends in Biochemical Science*. **23**: 198-199
2. Kerenyi Z, Merai Z, Hiripi L, Benkovics A, Gyula P, Lacomme C, Barta E, Nagy F, and Silhavy D. (2008) Inter-kingdom conservation of mechanisms of nonsense-mediated mRNA decay. *EMBO Journal*. **27**: 1585-1595
3. Hwang J, Sato H, Tang Y, Matsuda D, Maquat LE. (2010) UPF1 association with the cap-binding protein, CBP80, promotes nonsense-mediated mRNA decay at two distinct steps. *Mol Cell*. **39**: 396-409.
4. Heyer EE, and Moore MJ. (2016) Redefining the Translational Status of 80S Monosomes. *Cell*. **164**: 757–769
5. Sterne-Weiler T, Martinez-Nunez RT, Howard JM, Cvitovik I, Katzman S, Tariq MA, Pourmand N, Sanford JR (2013) Frac-seq reveals isoform-specific recruitment to polyribosomes. *Genome Res*. **23**: 1615-1623
6. Floor SN, and Doudna JA. (2016) Tunable protein synthesis by transcript isoforms in human cells. *eLife*. **5**: e10921