Revealing the hidden transcriptome: Analysis of nonsense-mediated mRNA decay targets reveal mechanistic insights

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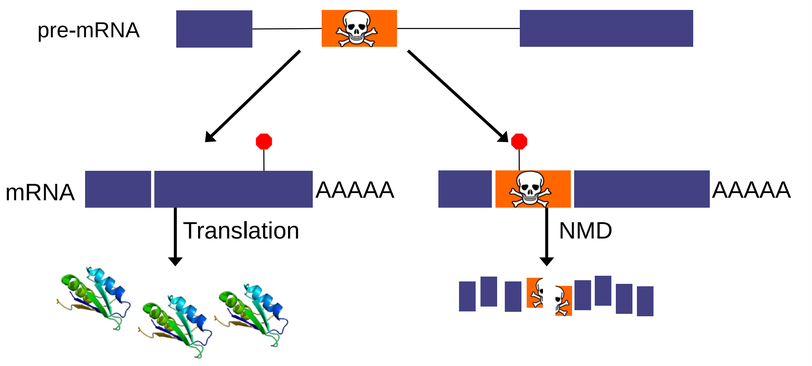
Alternative splicing coupled to nonsense-mediated mRNA decay regulates gene expression

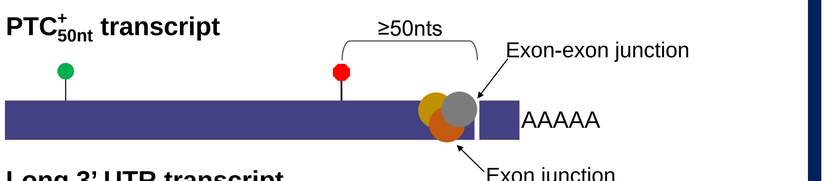
Alternative splicing coupled to nonsense-mediated mRNA decay (NMD) pathway regulates thousands of genes in human cells. NMD degrades transcripts with a premature termination codon (PTC) although the features that define a stop codon as premature are poorly understood. Here we use RNA-seq data from a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



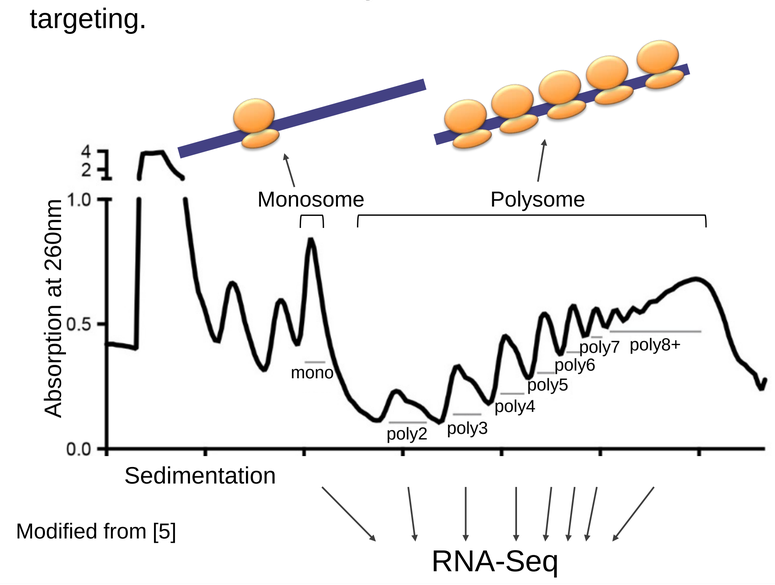
Goal

To understand what features define an NMD target in different species.

Using RNA-Seq to identify NMD targeted transcripts

NMD was inhibited in multiple species by knocking down or out UPF1, a core NMD factor. RNA-Seq was performed to identify transcripts with altered expression after UPF1 depletion.

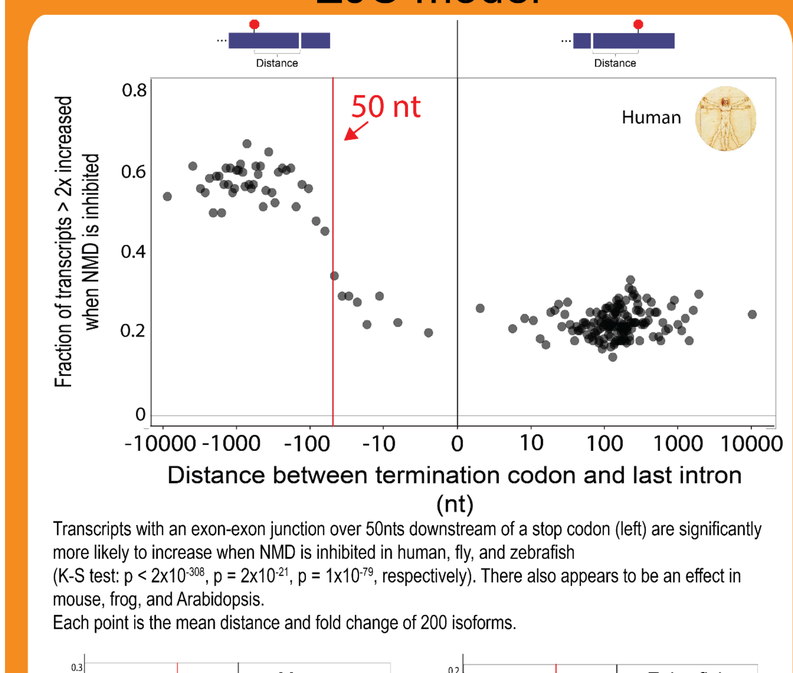
Transcripts bound by ribosomes can be separated into fractions on a mass gradient and then sequenced. NMD targets are known to be enriched in the monosome fraction (bound by a single ribosome) and depleted from the polysome fraction (bound by multiple ribosomes) in yeast and human cells [3,4]. Using polysome fraction data from human cells [5], 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.



Modified from [5]

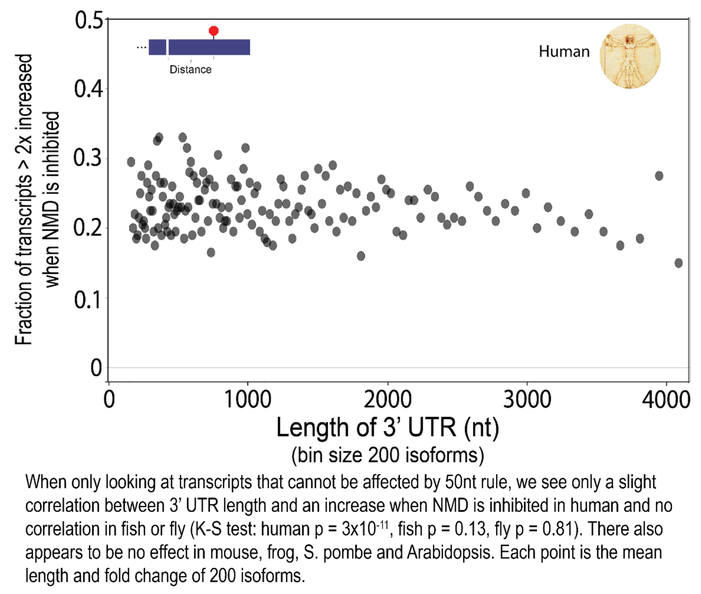
The EJC model is a strong predictor of NMD in humans and plays a role in other species while a longer 3' UTR has little to no effect in any species

EJC model



Transcripts with an exon-exon junction over 50nts downstream of a stop codon (left) are significantly more likely to increase when NMD is inhibited in human, fly, and zebrafish (K-S test: $p < 2x10^{-308}$, $p = 2x10^{-21}$, $p = 1x10^{-79}$, respectively). There also appears to be an effect in mouse, frog, and Arabidopsis.
Each point is the mean distance and fold change of 200 isoforms.

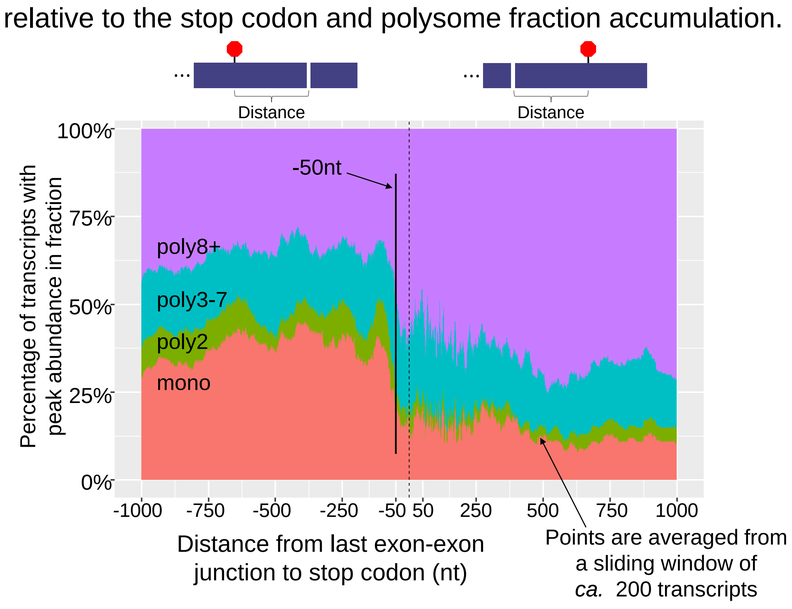
3' UTR length



When only looking at transcripts that cannot be affected by 50nt rule, we see only a slight correlation between 3' UTR length and an increase when NMD is inhibited in human and no correlation in fish or fly (K-S test: human $p = 3x10^{-11}$, fish $p = 0.13$, fly $p = 0.81$). There also appears to be no effect in mouse, frog, S. pombe and Arabidopsis. Each point is the mean length and fold change of 200 isoforms.

Transcripts with a PTC_{50nt} are enriched in the monosome fraction

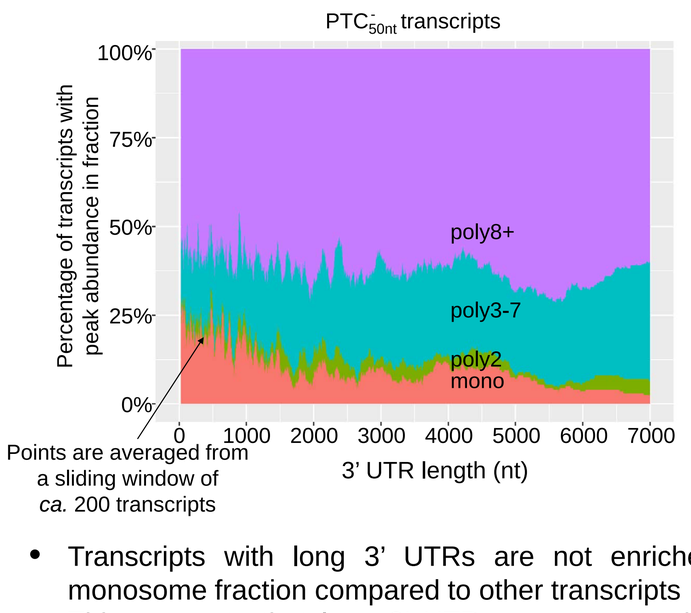
To test the EJC model for NMD targeting, we examined the relationship between the last exon-exon junction position relative to the stop codon and polysome fraction accumulation.



- Transcripts with a PTC_{50nt} are more likely to have peak abundance 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

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 and therefore not become abundant in the monosome fraction.

Conclusions

The EJC model is a strong predictor of NMD in human and also appears to have an often more limited role in numerous other species, except *S. pombe*.

3' UTR length has little correlation with NMD in any of the species checked.

Abundance of transcripts in the monosome fraction verses the polysome fractions confirms these trends in human cells without the need for perturbation of the NMD pathway.

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