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

We find that transcripts with PTCs 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.

What targets transcripts to NMD?

In mammals, the canonical model is that a PTC targets a transcript to NMD. A PTC 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].

<table>
<thead>
<tr>
<th>Percentage of transcripts with peak abundance in fraction</th>
<th>Transcripts with peak abundance in the poly8+</th>
<th>Transcripts with peak abundance in the poly7-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction of transcripts with peak abundance in the mono</td>
<td>Transcripts with peak abundance in the poly8+</td>
<td>Transcripts with peak abundance in the poly7-</td>
</tr>
<tr>
<td>Distance from stop codon to last exon-exon junction (nt)</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>Distance from last exon-exon junction to stop codon (nt)</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>Distance from stop codon to first exon-exon junction (nt)</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>Distance from first exon-exon junction to stop codon (nt)</td>
<td>0</td>
<td>1000</td>
</tr>
</tbody>
</table>

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 Flor and Doudna [6] to answer these questions.

Transcripts with a PTC50nt are enriched in the monosome fraction

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.

Conclusions

- Transcripts with a PTC50nt 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 GM102076 Jamie Cate) for funding James Lloyd, NDSEG Fellowship for funding Courtney French, the Tang Distinguished Scholarship to Gang Wei, and NIH R01-GM071655 to Steven Brenner. We thank Stephen Floor for advice on analysis of the polysome data.

References

6. Floor SN, and Doudna JA. Tunable protein synthesis by transcript isoforms in human cells. eLife. 2016. 5:e10921