Thousands of nonsense-mediated mRNA decay targets revealed by transcriptome analysis offer clues to the mechanism of degradation

Courtney E. French1*, Gang Wei2*, Angela N. Brooks1, and Steven E. Brenner1,2,$

1Department of Molecular and Cell Biology, 2Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3120, USA.

*These authors contributed equally to this work<br>

frenchrepl@compbio.berkeley.edu

INTRODUCTION:

Alternative splicing plays a major role in the generation of proteomic diversity. However, mistakes in this process can introduce a premature termination codon (PTC) and result in non-functional proteins that are harmful to the cell. Such transcripts are usually degraded by nonsense-mediated mRNA decay (NMD). The coupling of alternative splicing and NMD has also been reported as an important regulatory mechanism for certain sets of genes (3). Though many NMD targets have been identified in various species, we still lack a comprehensive view of the landscape of those transcripts degraded by NMD.

Here, we characterize the transcripts normally degraded by NMD in human Hela cells by inhibiting NMD through knockdown of the essential NMD factor UPF1 and performing RNA-seq analysis.

GOALS:

How many genes produce isoforms that are targets for NMD in human cells?

How high are transcript levels of RNAs that are targets for NMD?

What are the functional effects of NMD inhibition on the mRNA transcriptome?

How does NMD recognize early termination codons?

RESULTS:

A) Almost 2,800 robustly expressed transcripts, from a fifth of expressed genes, were identified as putative NMD targets.

B) Transcripts with a PTC were more likely to increase in abundance.

C) Many transcripts degraded by NMD are transcribed at high levels.

A PTC (50nt) is a strong predictor of degradation through NMD while a 3' UTR has little effect.

NMD-targeted transcripts are validated by qPCR.

CONCLUSIONS:

- Over 2,700 robustly expressed isoforms from over 2,100 genes (19% of expressed genes) are degraded by NMD.
- 1,548 of NMD-targeted transcripts are novel isoforms (55%).
- A rule for termination-codon position within intron-containing genes: when nonsense affects RNA splicing.
- Genes from many other functional categories also produce NMD targets.
- Transcripts targeted by NMD are significantly enriched for exonic ultraconserved elements.
- Coupling of alternative splicing and NMD appears to regulate the expression of hundreds of genes.
- There is strong support for the 50nt rule in NMD degradation in human cells.

ACKNOWLEDGEMENTS:

The fly RNA-seq knockout data set was generated by the modENCODE Fly Transcriptome Group (Sara Celhrkoi, Brenton Gravely, Steven Brenner, Gemma May, Li Yang, Angela Brooks). Read alignments were done by Mike D'Asato, Sandrine Dutout, Kaspar Hansen. We thank Adam Roberts and Lisa Pachter of UC Berkeley for help with the optimization of Culbinska. This work was funded by an NIH Grant R01 GM071513 to S.R.B., C.E.F, was supported by a Tang Distinguished Scholarship from QB3 at UC Berkeley, and C.E.F. was supported by an NIH 5G32 GM075717 grant.

REFERENCES:


