INTRODUCTION:

Non-mRNA-mediated mRNA decay (NMD) is an RNA surveillance system that removes aberrant isoforms containing a premature translation termination codon. This pathway is conserved throughout eukaryotes and protects against the production of harmful truncated proteins. Additionally, NMD coupled with alternative splicing is a mechanism of post-transcriptional gene regulation that affects the mRNA levels of hundreds of genes in human(1). The canonical model of defining a premature termination codon in mammals is the 50nt rule: a termination codon more than 50 nucleotides upstream of an exon-exon junction is premature and triggers NMD. In other animals, a 3' UTR intron is not required for NMD. There is also evidence that a longer 3' UTR triggers NMD in plants, flies, and mammals(2). The importance of each mechanism appears to vary between species, and it is currently unclear which is the major mechanism at work in human cells. We used RNA-seq analysis done on cells with inhibited NMD to determine the features associated with degradation in human and in fly.

GOALS:

How conserved are the targets of alternative splicing coupled with NMD?
What features define a premature termination codon in different species?

APPROACH:

1. Control RNA-seq library preparation.
2. NMD inhibition through knockdown/knockout of UPF1.
3. High-throughput Illumina sequencing.
4. Transcript assembly and quantification with Cufflinks [5] or JunoBASE [6].
5. Directional and paired-end RNA-seq library preparation.
6. Map reads to genome with TopHat [4].
7. Premature translation termination codon prediction.

REFERENCES:


CONCLUSIONS:

Thousands of alternatively spliced genes (>20%) produce transcripts that fall into our strict set of NMD targets in human.

Hundreds to thousands of alternatively spliced genes (11-42%) produce transcripts possibly degraded by NMD in diverse eukaryotes.

The 50nt rule 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.

HUNDREDS TO THOUSANDS OF GENES PRODUCE ALTERNATIVE ISOFORMS DEGRADED BY NMD

Since an intron sufficiently downstream of the termination codon is known to trigger NMD (50nt rule), we defined a strict set of probable NMD targeted genes that are required to have an isoform that follows the 50nt rule (contains a 3' UTR exon) and increases >2x (after controlling for transcriptional changes) when NMD is inhibited. Over 20% of alternatively spliced genes are targeted by NMD in human (RED). S. pombe was excluded from this analysis as the 50nt rule doesn’t seem to play a role in that species, unlike the others (see below).

The 50nt rule is a strong predictor of NMD in human and plays a role in other species while a longer 3' UTR has little to no effect in any species.