Transcriptome-wide investigation of stop codon readthrough in Saccharomyces cerevisiae [poster]

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Translation of mRNA into a polypeptide is terminated when the release factor eRF1 recognizes a stop codon in the ribosomal A site and stimulates nascent peptide release. However, “stop codon readthrough” can occur when a near-cognate tRNA outcompetes eRF1 in decoding the stop codon, resulting in the continuation of elongation into the mRNA 3′-UTR. Previous studies with reporter systems have shown that the efficiency of termination or readthrough is modulated by multiple cis-acting elements (Figure 1). It remains to be determined whether these elements are important at a genome-wide level and whether other mRNA features proximal to the stop codon significantly affect termination and readthrough efficiencies in vivo. Accordingly, we carried out ribosome profiling analyses of yeast cells expressing defective or depleted eRF1 and developed bioinformatics strategies to calculate readthrough efficiency, and to identify mRNA and peptide features which influence that efficiency.

Ribosome profiling datasets

eRF1 (sup45 for yeast) mutants:  
- This project  
- WT at 25 °C  
- WT at 37 °C  
- sup45-ts at 25 °C  
- sup45-ts at 37 °C  
- Wu et al., Molecular Cell, 2019  
- WI  
- sup45-depleted

Recycling factors mutants:  
- to rule out effects of re-initiation events  
- Young et al., Cell, 2015  
- WT  
- WT  
- Young and Guydosh, Cell Rep, 2019  
- WT  
- htr1A

Calculation of readthrough efficiency

A

B

Figure 2. A. Schematic depicting ribosome footprint count across a gene’s regions (Adapted from Brar and Weissman, Nat. Rev. Mol. Cell Biol., 2015), B. Formula to calculate readthrough efficiency.

Analysis strategies

Two machine learning approaches involving the random forest algorithm (Breiman, Mach. Learn., 2001) were trained for each sample:  
1. Regression: use X to predict Y.  
2. Classification: use X to predict Y.

The remaining genes were sorted by their readthrough efficiency values and grouped:

Random forest analyses determined that stop codon identity, P-site amino acid identity, and 3′-UTR length are important factors in predicting readthrough efficiency.

Identities of readthrough-permissive stop codons and nucleotides at positions -2 to +9 were mostly true at a genome-wide level

Specific codons in the P site were associated with higher or lower readthrough efficiencies

Readthrough efficiency increased with 3′-UTR lengths in cells expressing defective or depleted eRF1, but not in wild-type or recycling factor mutant cells

CONCLUSIONS

Our results confirmed the general roles of known regulatory elements in genome-wide regulation and identified several new mRNA or peptide features important for translation termination and readthrough:

- Random forest analyses revealed that the most influential features consist of the stop codon, the penultimate codon in the P site, and 3′-UTR length.
- Known effects of stop codon and surrounding nucleotide identities were mostly true at a genome-wide level.
- Specific codons in the P site were associated with higher (CUG, CUU, AUA, ACA, ACC) or lower (UUA, CGU, GCC) readthrough efficiencies.
- Readthrough efficiency increased with 3′-UTR length (implying proximity to PABP) in eRF1 mutant cells, while this trend was reversed in wild-type cells, suggesting that PABP’s expected role in aiding termination was prominent only when eRF1 was inefficient. This is consistent with the extents of premature termination or readthrough observed in three different genes in sup45Δ cells (Abstract/Talk: Chan Wu, et al.).