INVESTIGATING THE DYNAMICS OF TRANSLATION READTHROUGH IN LIVE CELLS

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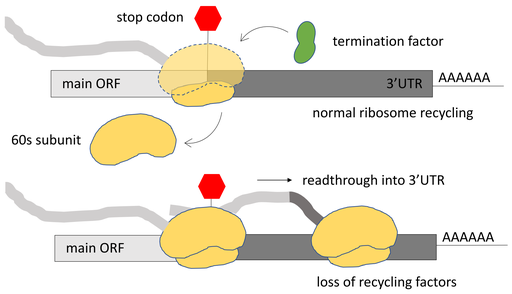
The possible mechanisms by which translational readthrough occur

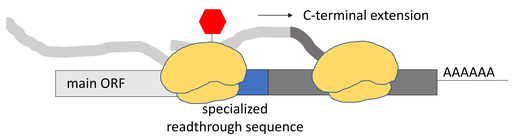
1 Termination factor depletion can lead to ribosomes in the 3'UTR



Ribosomes are recycled at stop codons with the help of termination factors.

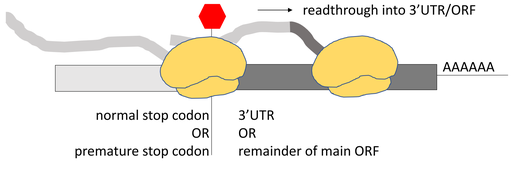
When those are depleted, ribosomes undergo readthrough[1].

2 Some genes have programmed readthrough signals



Some genes encode readthrough sequences to make C-terminal extensions[2].

3 Treatment with aminoglycosides can induce readthrough of both normal and premature stop codons

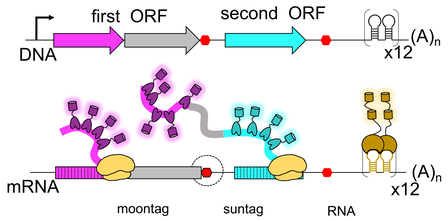


Aminoglycoside antibiotics bind to ribosomes to inhibit protein synthesis.

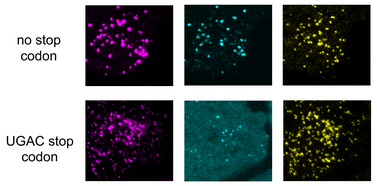
Many human genetic diseases are caused by premature stop codons within the coding region[3]. Treatment of cells with aminoglycoside antibiotics induces readthrough at both premature and normal stop codons[4].

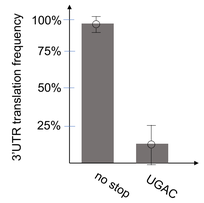
Understanding the factors that lead to normal stop codon recognition vs those that lead to translational readthrough of stop codons has therapeutic potential.

Single molecule reporters allow us to directly visualize readthrough in real time



Moontag/Suntag[5-7] are repeat epitopes recognized by fluorescent nanobodies. RNA is visualized using PP7 stem loops and fluorescently-labelled coat proteins.



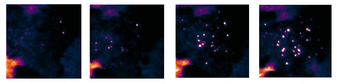


Why use single molecule reporters?

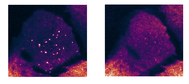
Can directly correlate multiple simultaneous signals over time.

We can **detect 3'UTR translation events** and see differences between stop codon contexts.

Controls to verify translation status of signals



inducing expression of reporter shows gradual increase in translation events



translation inhibition removes ribosomes from mRNA

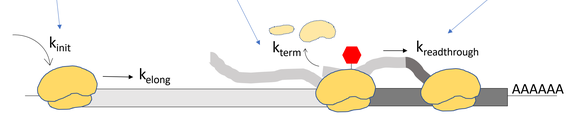
Different stop codons have unique readthrough propensities[8]. Can we detect this using our single molecule reporters?

Modeling different readthrough mechanisms via stochastic simulations

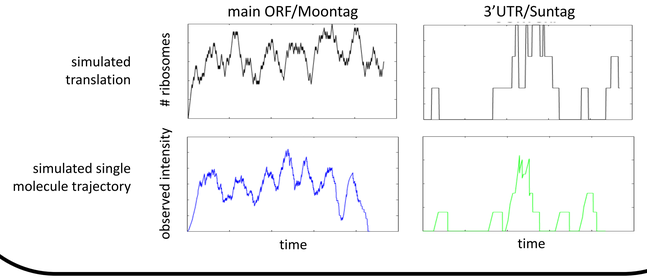
initiation rate = how many ribosomes per minute start translating the main ORF

Termination rate = based on how fast ribosomes translate the main ORF

Readthrough rate = how often ribosomes bypass the stop codon

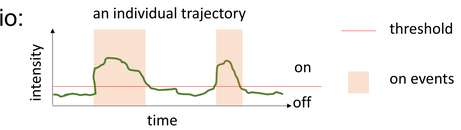


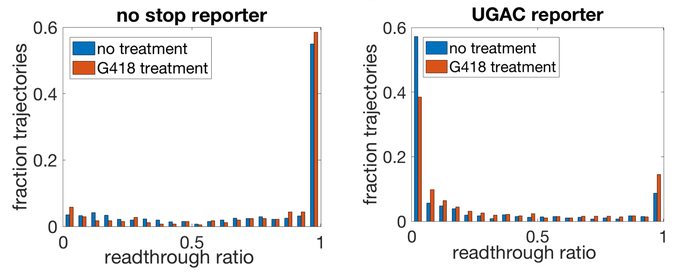
Stochastic modeling can simulate how translation occurs for different readthrough models. We can then use the simulations to make simulated single molecule traces and see which model most accurately represents our data.



Comparing real data to simulations suggests "burst-like" readthrough

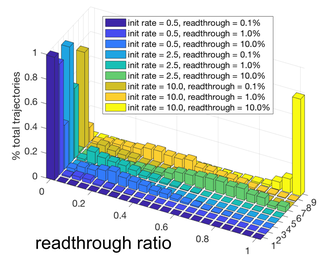
: readthrough ratio: $\frac{(\Delta t \text{ readthrough})}{(\text{total traj len})}$





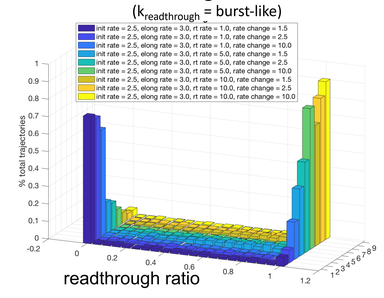
The UGAC reporter shows low levels of readthrough, with readthrough either occurring or not for the trajectory duration. It increases with aminoglycoside treatment as expected.

Stochastic readthrough model ($k_{readthrough}$ = constant)



Stochastic readthrough model shows a single, Gaussian like distribution

Bursting model ($k_{readthrough}$ = burst-like)



A bursting readthrough model shows a bimodal distribution where translation is either off or on.

Comparison of the data to simulations suggests a "bursting" model of translational readthrough.

Future experiments

1 **Investigate more stop codon contexts, including programmed stop codon sequences and premature stop codon contexts**

no stop codon | Programmed stop codon seq's | UGAC stop codon | UAGC stop codon | UAAA stop codon | multiple stop codons

stop codon "leakiness"

2 **Knockdown recycling factors to determine each factor's contribution to readthrough dynamics**

Knocking down recycling factors and using our single molecule assays can help uncover how these recycling factors influence readthrough dynamics, which in turn can inform us on readthrough mechanisms.

3 **Investigate the possible determinants of bursting behavior**

What causes readthrough bursting? Possibilities include RNA structure, modifications, or RNA-binding proteins.

References and Acknowledgements

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We would like to thank Dr. Marvin Tanenbaum for the gift of Moontag-containing cell strains. Additionally, thanks to Agnes Karasik for development of reporters used in this poster, and initial data collection and data collection optimization.