Unconventional mRNA silencing in baker's yeast: junk to gold?

Paolo L. Sinopoli[1], Regina T. Nostramo[1 2], Alicia Bao[3], Sara Metcalf[1], and Anita K. Hopper[1 2]

[1]Department of Molecular Genetics, [2]Center for RNA Biology, [3]College of Medicine, The Ohio State University, Columbus, OH, USA

Department of MOLECULAR GENETICS

THE OHIO STATE UNIVERSITY — CENTER FOR RNA BIOLOGY

Abstract

Within cells, genetic code (DNA) is converted into messengerRNAs (mRNAs) through a process called transcription. mRNAs then function as a template for ribosomes to build the proteins responsible for nearly every aspect of cellular life. Protein creation is heavily regulated, and cells utilize various methods to strictly control protein levels. One of these methods is the suppression of mRNAs, known as mRNA silencing. Through mRNA silencing, specific mRNAs may become non-functional in protein creation and be targeted for degradation. Traditionally, mRNA silencing takes place through a pathway involving two specific proteins: argonaute and dicer. While advanced organisms like humans possess both proteins, some organisms, such as baker's yeast, lack these proteins, and were therefore thought to not undergo mRNA silencing. However, **we have identified a new pathway of mRNA silencing in baker's yeast that functions via transferRNA introns.** TransferRNAs (tRNAs) act to transfer amino acids to the ribosome in order for proteins to be synthesized. When created, some tRNAs contain an intron that must be removed for the tRNA to gain functionality. It is this removed region, the tRNA intron (which was previously thought to have little biological function), that we have identified to suppress mRNAs in baker's yeast. To discover this, we employed bioinformatics in conjunction with biological methods involving the complete removal of the tRNA^{Ile} introns from the genetic code of baker's yeast.

Background

Proteins are made through the translation of mRNAs.

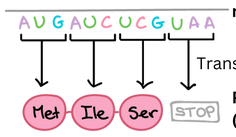
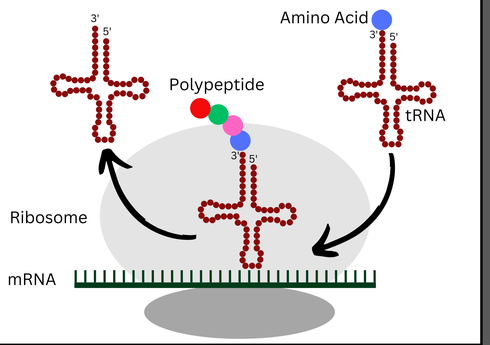
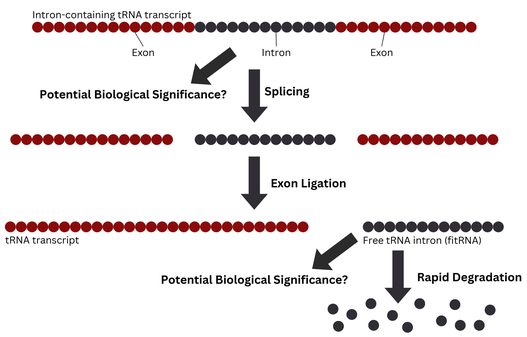


Figure adapted from Khan Academy CC BY 4.0

tRNAs are non-coding RNAs that function in protein synthesis.



Some tRNA genes contain introns, which are spliced and rapidly degraded.



Hypothesis

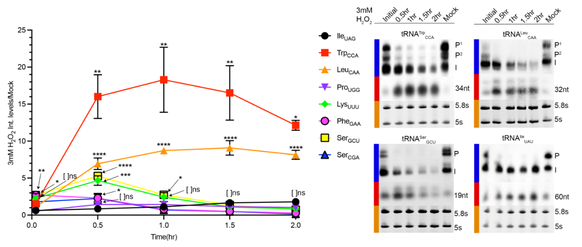
tRNA introns are biologically significant and act to regulate gene expression.

Presence of tRNA introns is highly conserved

All eukaryotic organisms listed on the tRNA genome database have at least one intron-containing tRNA family.

tRNA introns accumulate in a family and stress specific manner

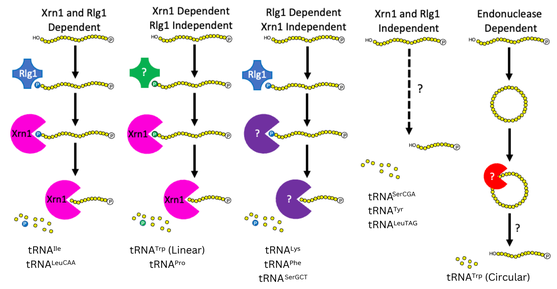
In baker's yeast, free introns from different tRNA families accumulate to varying degrees under oxidative stress induced by H_2O_2.



Sara Metcalf

tRNA introns have multiple degradation pathways

In baker's yeast, all tRNA introns are spliced by the SEN complex at the mitochondria, releasing free tRNA introns. A minimum of five different family-specific pathways then work to rapidly degrade free introns.

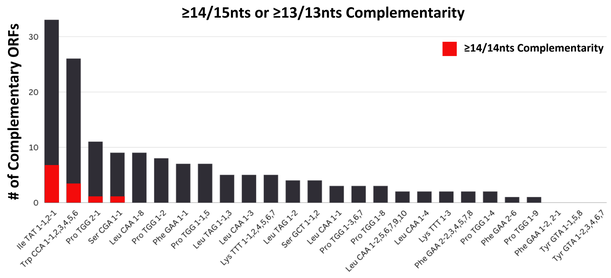


Rlg1/Trl1: tRNA ligase known to phosphorylate tRNA introns.
Xrn1: 5' to 3' exonuclease known to degrade phosphorylated tRNA introns.

Sara Metcalf and Alicia Bao

tRNA intron complementarity to mRNA coding regions in baker's yeast lacks uniformity

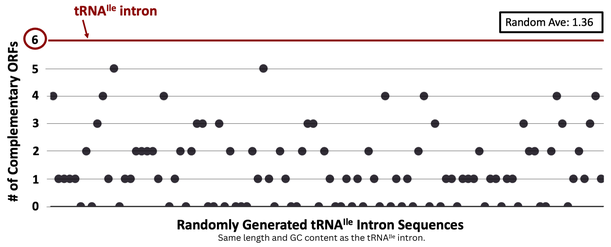
In baker's yeast there are 26 unique tRNA intron sequences across 10 intron-containing tRNA families. The number of complementary coding ORFs (Open Reading Frames) varies widely between intron sequences.



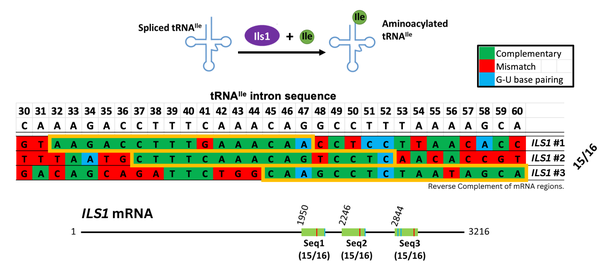
tRNA intron sequences have exceptional levels of complementarity to mRNA coding regions

The tRNA^{Ile} intron has ≥14/14nts of perfect complementarity to **six** ORFs.

Out of 100 randomly generated tRNA^{Ile} intron sequences (●), **none** have complementarity to as many coding ORFs as the **tRNA^{Ile} intron.**



When allowing for G-U base pairing, the tRNA^{Ile} intron has three unique regions of 15/16nts complementarity to tRNA^{Ile} synthetase Ils1.



Probability of three unique sixteen nucleotide sequences appearing in *ILS1* (allowing for G-U base pairing and one mismatch):

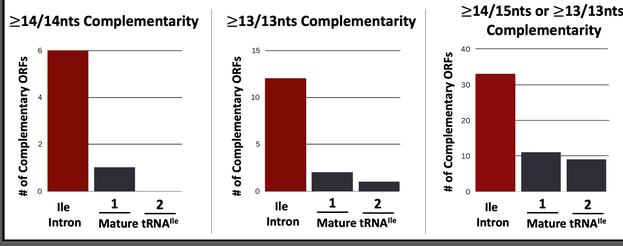
1 / [(4^{16})^3 / (Number of sequence possibilities due to mismatch * G-U pairing possibilities * Size of *ILS1*)^3]

1/8,600,000,000

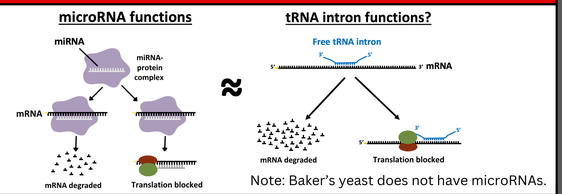
tRNA complementarity to mRNA coding regions is localized to the tRNA introns

The **tRNA^{Ile} intron** has more ORFs with complementarity than the **tRNA^{Ile} exons.**

tRNA^{Ile} Intron: 60 nucleotides
Mature tRNA^{Ile}: 73 nucleotides



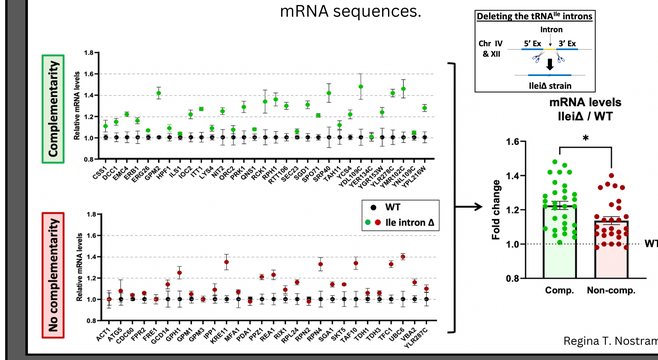
tRNA introns may act similar to the microRNAs that exist in other organisms



Note: Baker's yeast does not have microRNAs.

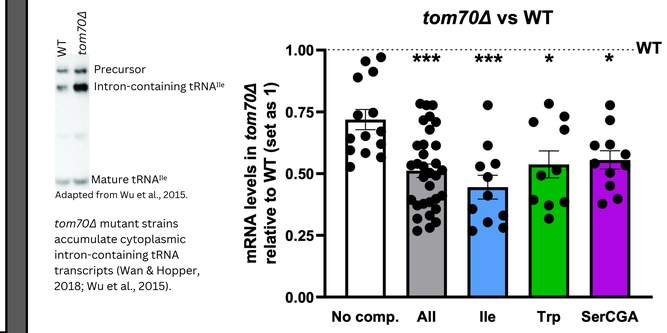
tRNA introns have an inhibitory effect on mRNA levels of genes with complementarity

Deletion of the tRNA^{Ile} intron results in a statistically significant increase in complementary mRNA sequence levels compared to non-complementary mRNA sequences.



Regina T. Nostramo

Deletion of *TOM70* leads to decreased levels of mRNA sequences with complementarity to various tRNA introns.



Adapted from Wu et al., 2015.

tom70Δ mutant strains accumulate cytoplasmic intron-containing tRNA transcripts (Wan & Hopper, 2018; Wu et al., 2015).

Accumulation of intron-containing tRNAs results in statistically significant decreases in levels of mRNA sequences with complementarity to tRNA introns compared to sequences without complementarity.

Regina T. Nostramo

Model and Conclusions

Intron-containing tRNAs and/or free tRNA introns act in a regulatory manner to suppress gene expression via sequence complementarity to mRNAs.

Figure made in BioRender

Acknowledgements

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References

Wan, Y., & Hopper, A. K. (2018). From powerhouse to processing plant: conserved roles of mitochondrial outer membrane proteins in tRNA splicing. Genes & development, 32(19-20), 1309–1314. https://doi.org/10.1101/gad.316257.118

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