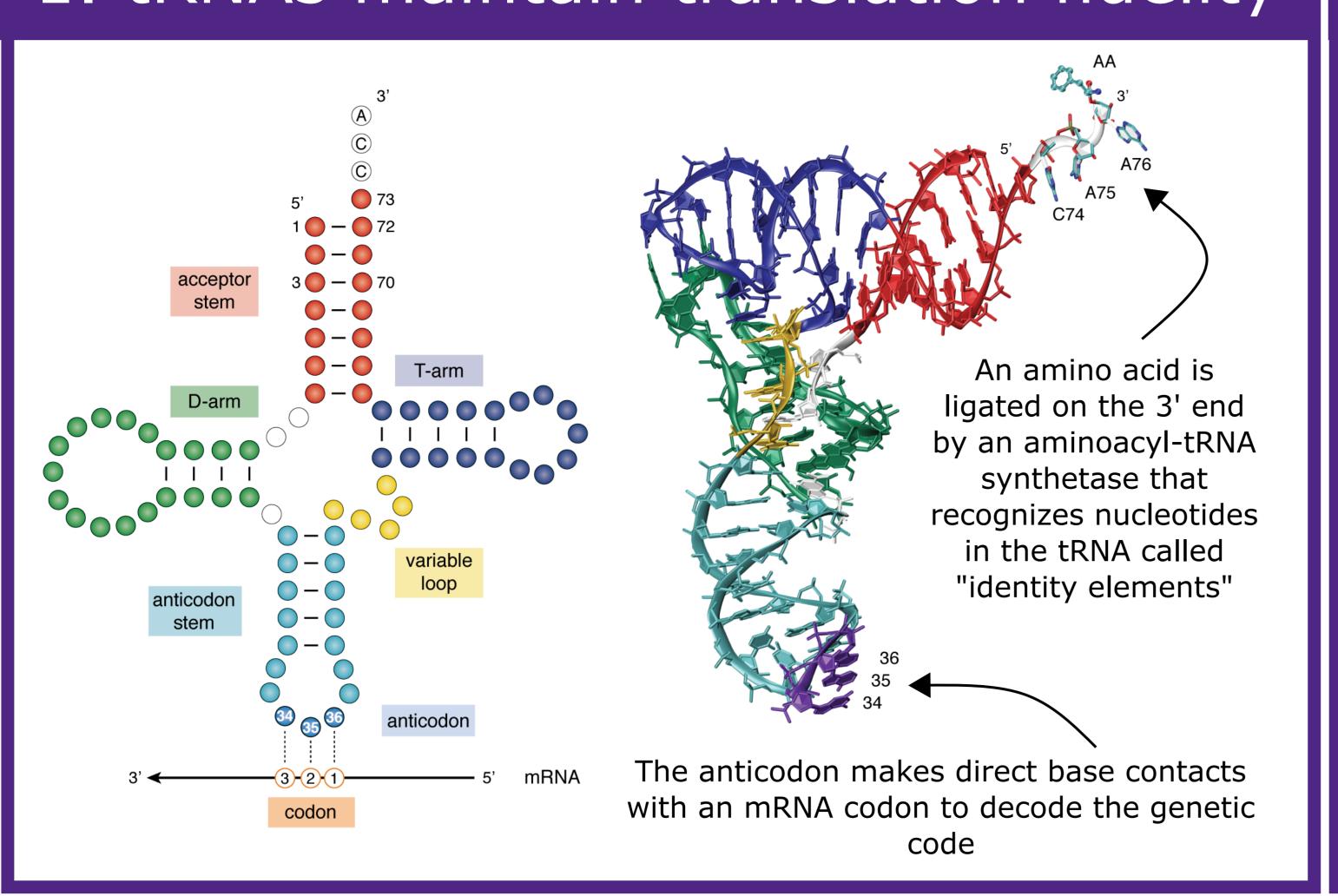# Mistranslation elicits different cellular responses based on the amino acid substitution
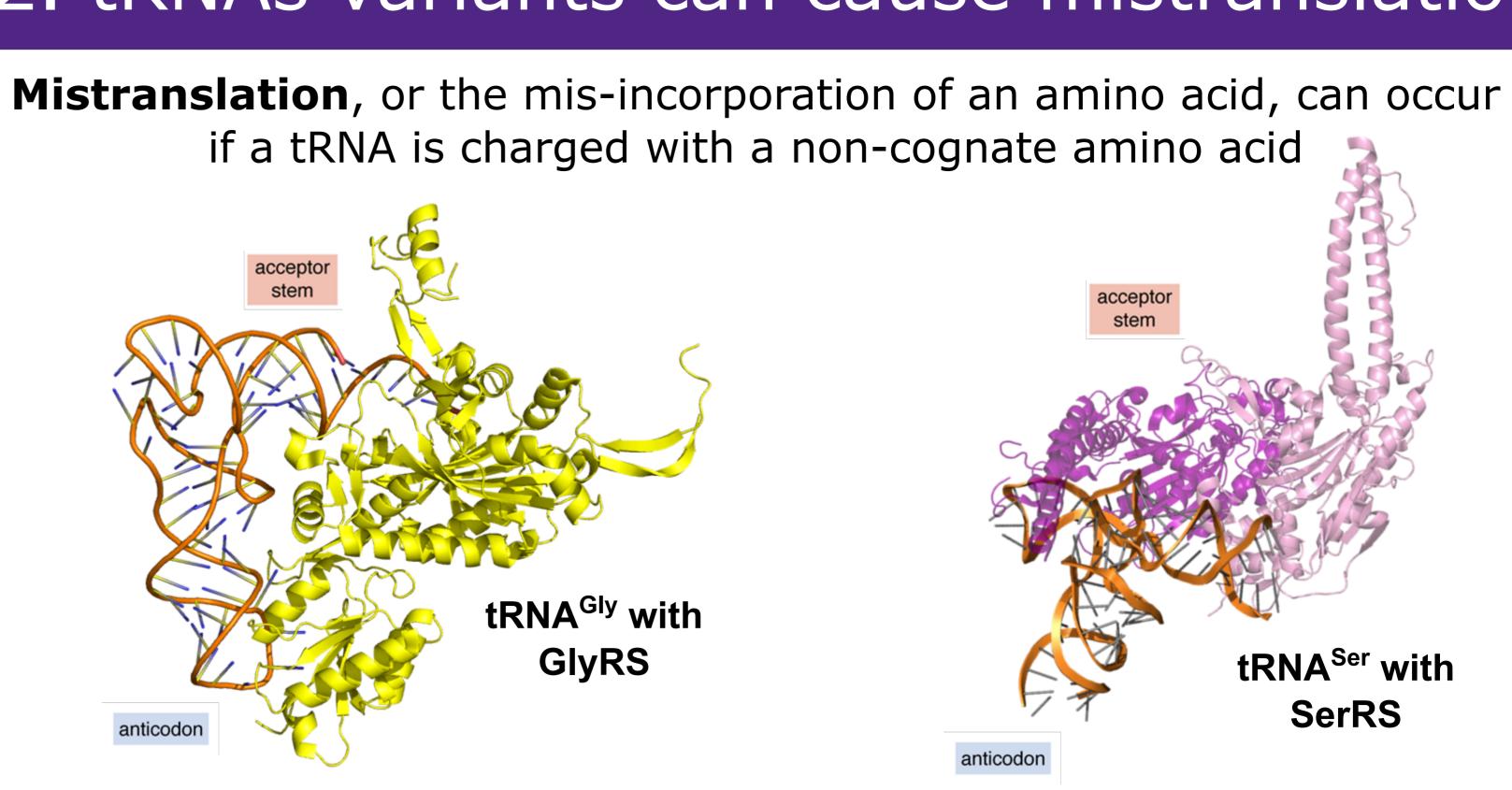
Matthew Berg[1], Yanrui Zhu[1], Bianca Y. Ruiz[2], Joshua Isaacson[1], Julie Genereaux[1], Raphael Loll-Krippleber[3], Bryan-Joseph San Luis[3], Charles Boone[3], Grant W. Brown[3], Judit Villen[2] and Christopher Brandl[1]

[1]Department of Biochemistry, The University of Wetsern Ontario, London, Canada [2]Department of Genome Sciences, University of Washington, Seattle, USA [3]Donnelly Centre for Cellular and Biomolecular Research, Univeristy of Trotonto, Toronto, Canada

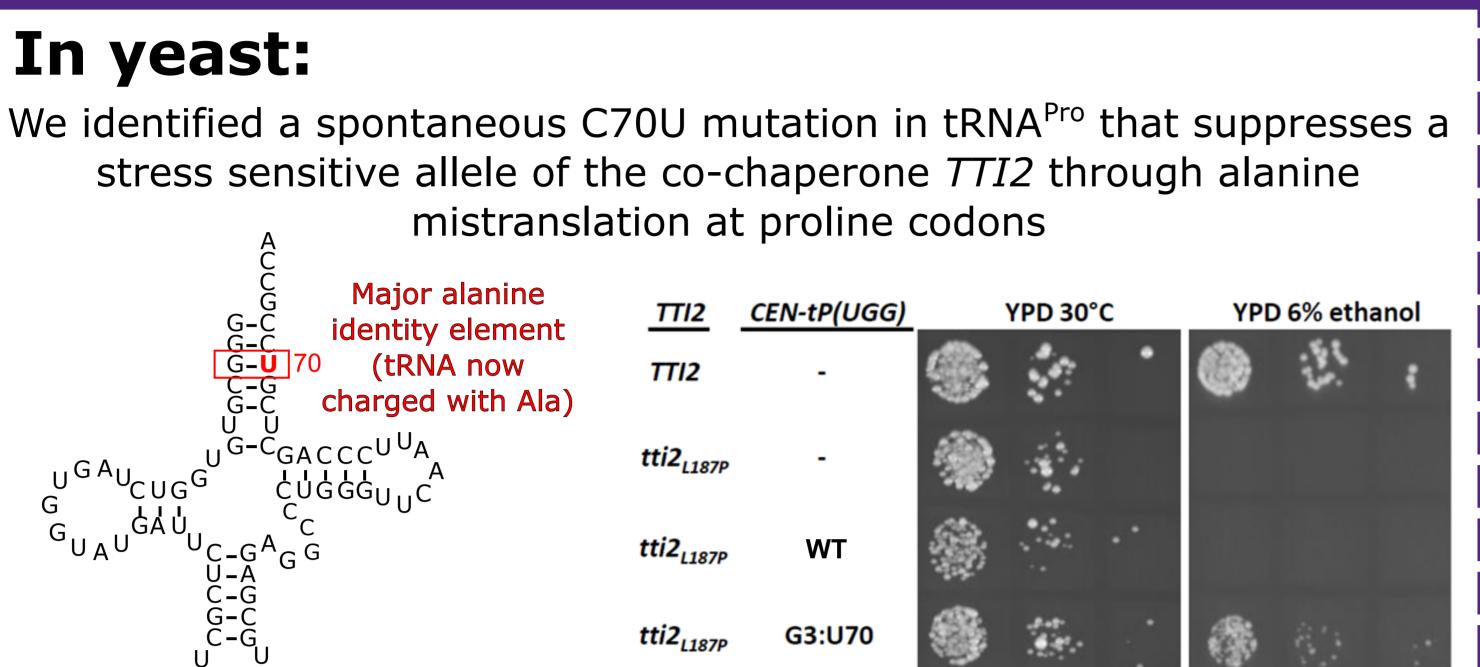## 1. tRNAs maintain translation fidelity

An amino acid is ligated on the 3' end by an aminoacyl-tRNA synthetase that recognizes nucleotides in the tRNA called "identity elements"

The anticodon makes direct base contacts with an mRNA codon to decode the genetic code

2. tRNAs variants can cause mistranslation

Mistranslation, or the mis-incorporation of an amino acid, can occur if a tRNA is charged with a non-cognate amino acid

tRNA[Gly] with GlyRS

tRNA[Ser] with SerRS

Most aminoacyl-tRNA synthetases recognize their cognate tRNAs through direct contacts with the tRNA anticodon

For tRNA[Ser] and tRNA[Ala] in yeast, the aminoacyl-tRNA synthetase does not recognize the anticodon

3. Naturally occuring mistranslating tRNA variants

In yeast:

We identified a spontaneous C70U mutation in tRNA[Pro] that suppresses a stress sensitive allele of the co-chaperone *TTI2* through alanine mistranslation at proline codons

Major alanine identity element (tRNA now charged with Ala)

Wild-type proline anticodon

TTI2	CEN-tp(UGG)	YPD 30°C	YPD 6% ethanol
TTI2	-		
tti2[L187P]	-		
tti2[L187P]	WT		
tti2[L187P]	G3:U70		

In humans:

From a group of 84 individuals, we identified 24 tRNA variants that alter tRNA anticodons or change tRNA identity elements

tRNA-Ser-AGA-2-3
chr6:27463627 G/A
AF = 3%

Serine misincorporation at phenylalanine codons
*Predicted mistranslation

tRNA-Gly-GCC-1-5
chr21:18827175 A/G
AF = 1.2%
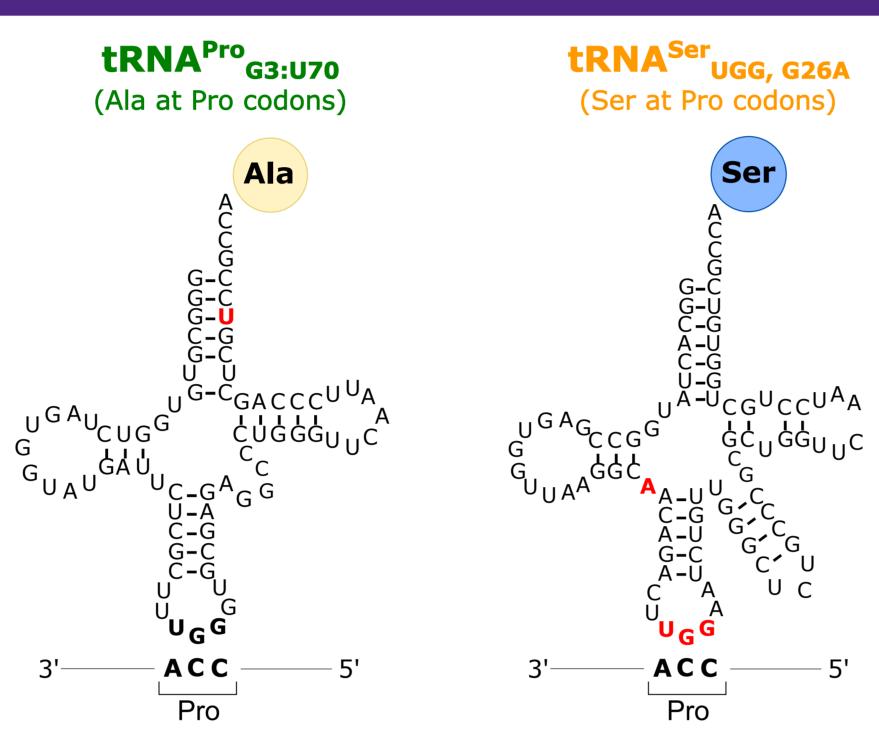
Alanine misincorporation at glycine codons
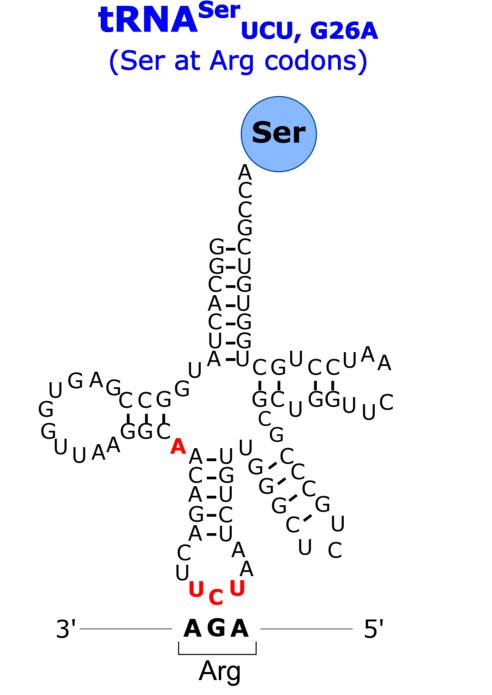*Predicted mistranslation

4. Do different frequencies and types of mistranslation have diffferent impacts?
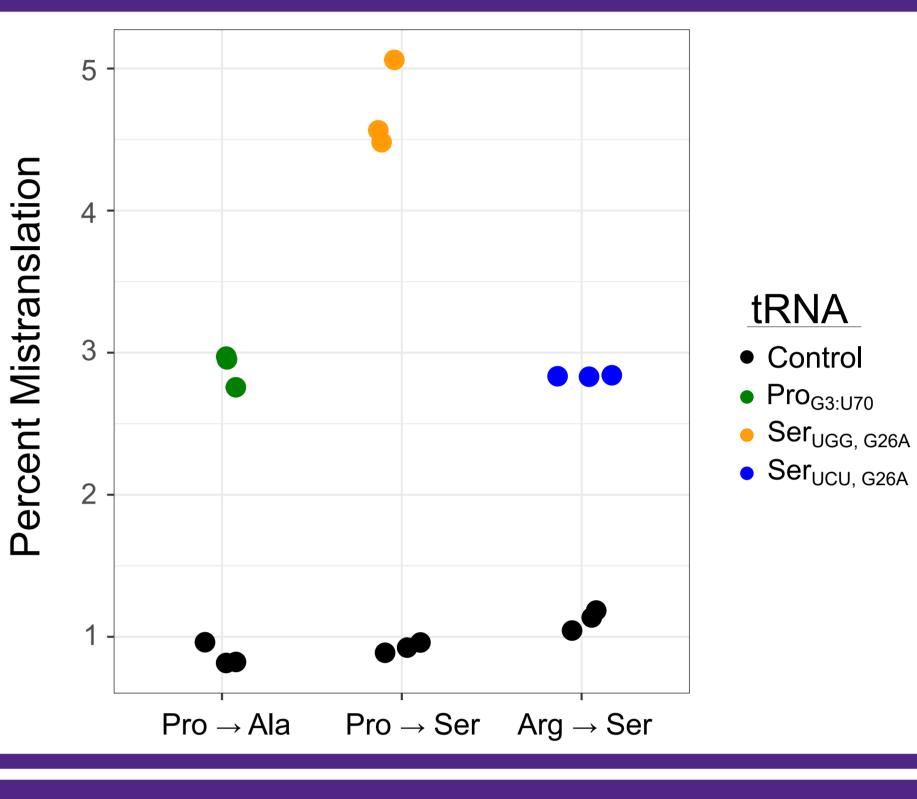
GOAL: To assess the response of yeast to mistranslation caused by different tRNA variants that differ in extent of mistranslation and the substitution they cause.
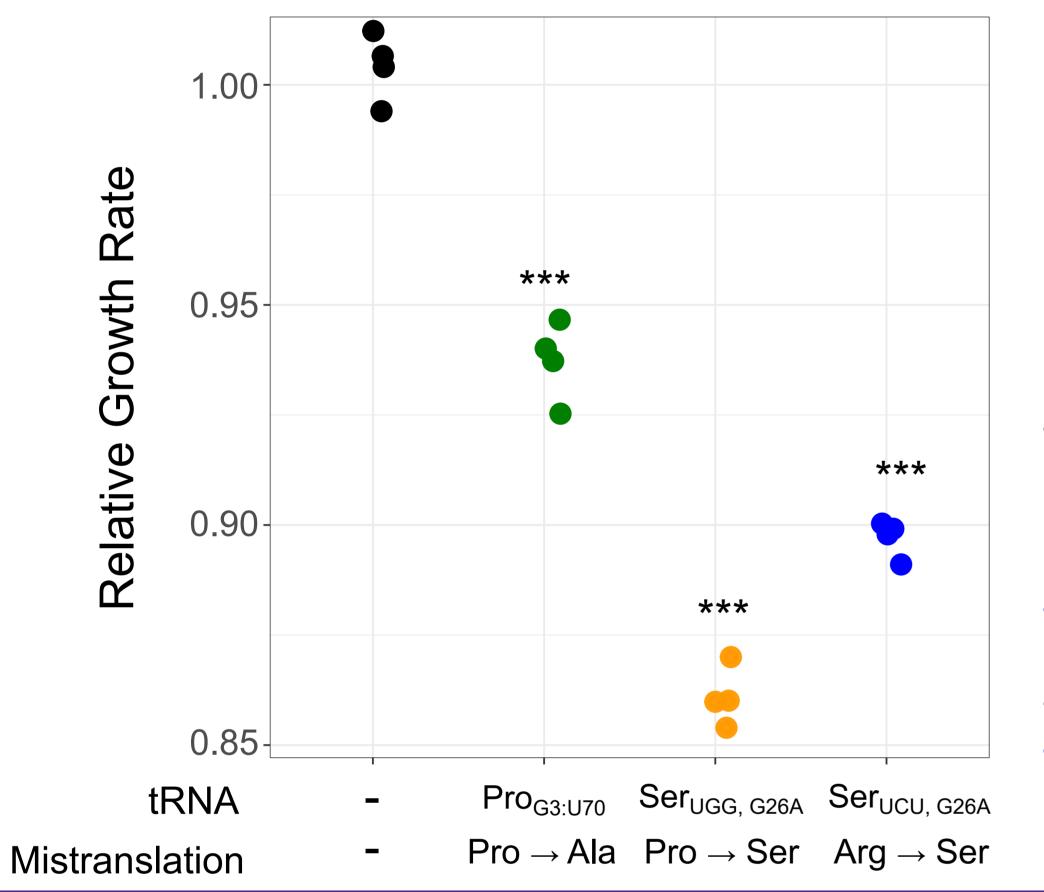
tRNA[Pro] G3:U70 (Ala at Pro codons)
tRNA[Ser] UGG, G26A (Ser at Pro codons)
tRNA[Ser] UCU, G26A (Ser at Arg codons)

Percent Mistranslation

tRNA: Control, Ser[G3:U70], Ser[UGG, G26A], Ser[UCU, G26A]

Pro → Ala, Pro → Ser, Arg → Ser

5. Mistranslation effect on growth

Relative Growth Rate

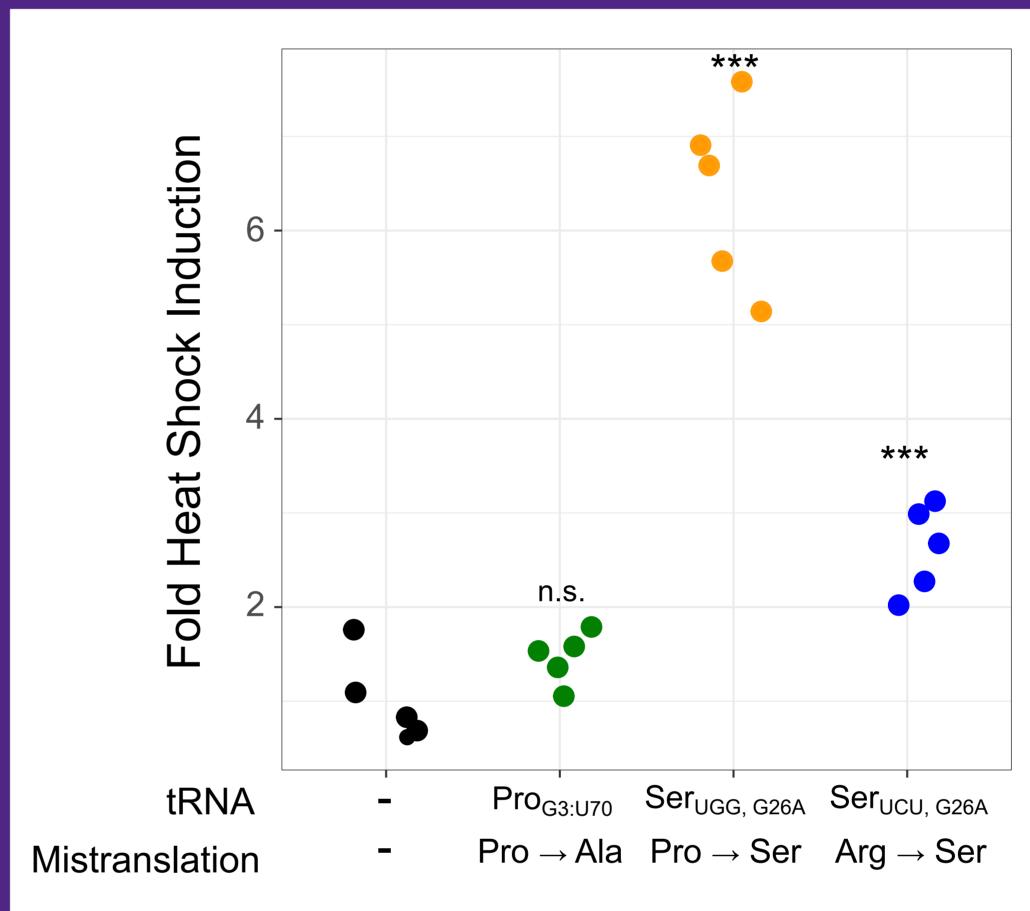| tRNA | - | Pro[G3:U70] | Ser[UGG, G26A] | Ser[UCU, G26A] |
| Mistranslation | - | Pro → Ala | Pro → Ser | Arg → Ser |

To determine growth rate, we calculated the relative growth rate of each tRNA-containing strain in liquid media

• tRNA[Ser] UGG, G26A mistranslates at the highest frequency and has the most severe effect on growth

• tRNA[Pro] G3:U70 and tRNA[Ser] UGG, G26A mistranslate at similar frequencies, but tRNA[Ser] UCU, G26A has a greater effect on growth

6. Mistranslation effect on heat shock

Fold Heat Shock Induction

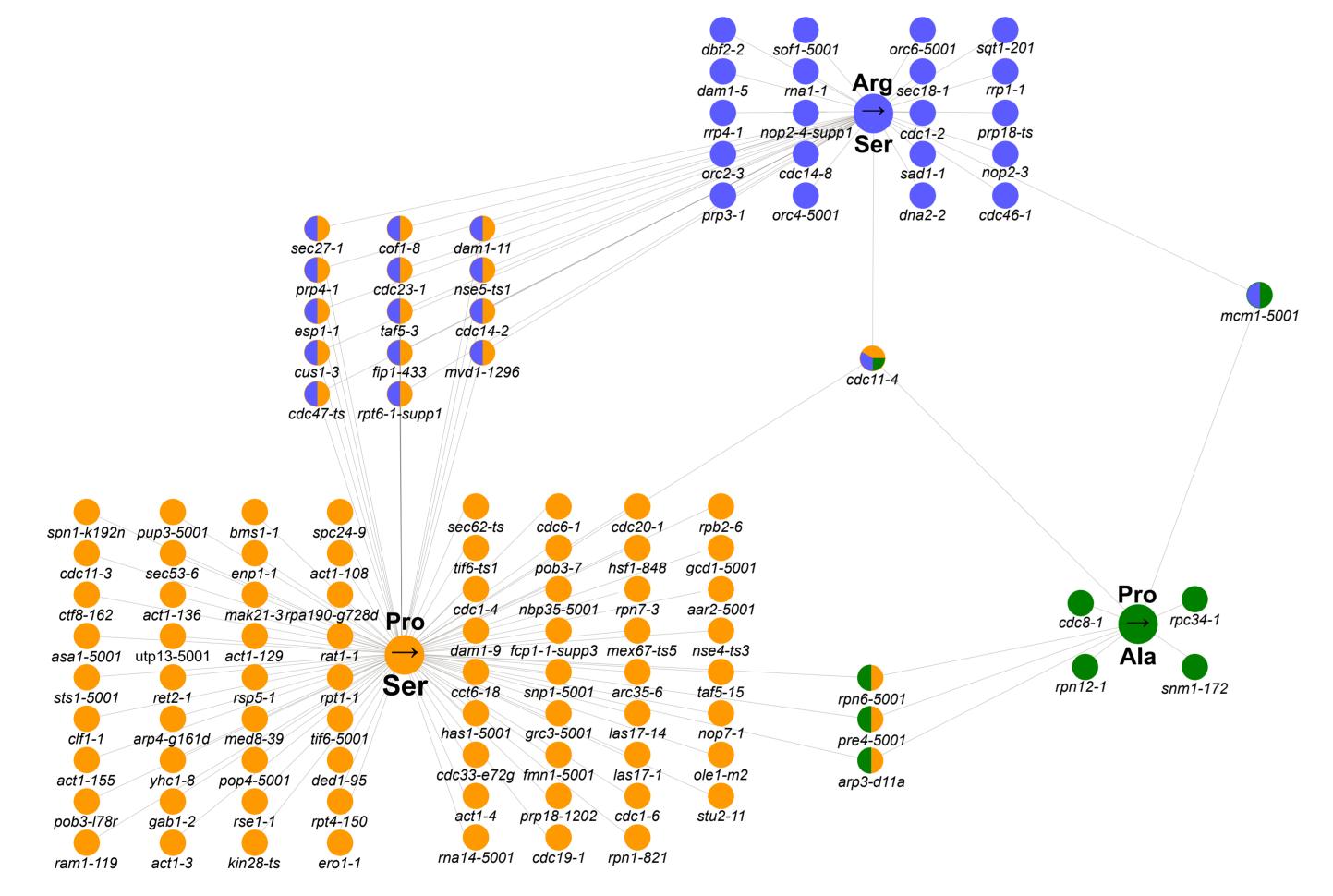| tRNA | - | Pro[G3:U70] | Ser[UGG, G26A] | Ser[UCU, G26A] |
| Mistranslation | - | Pro → Ala | Pro → Ser | Arg → Ser |

To determine the heat shock response induced by each tRNA, we used a GFP reporter driven by heat shock response elements

• Similar to the growth results, tRNA[Ser] UGG, G26A induced the greatest heat shock reponse
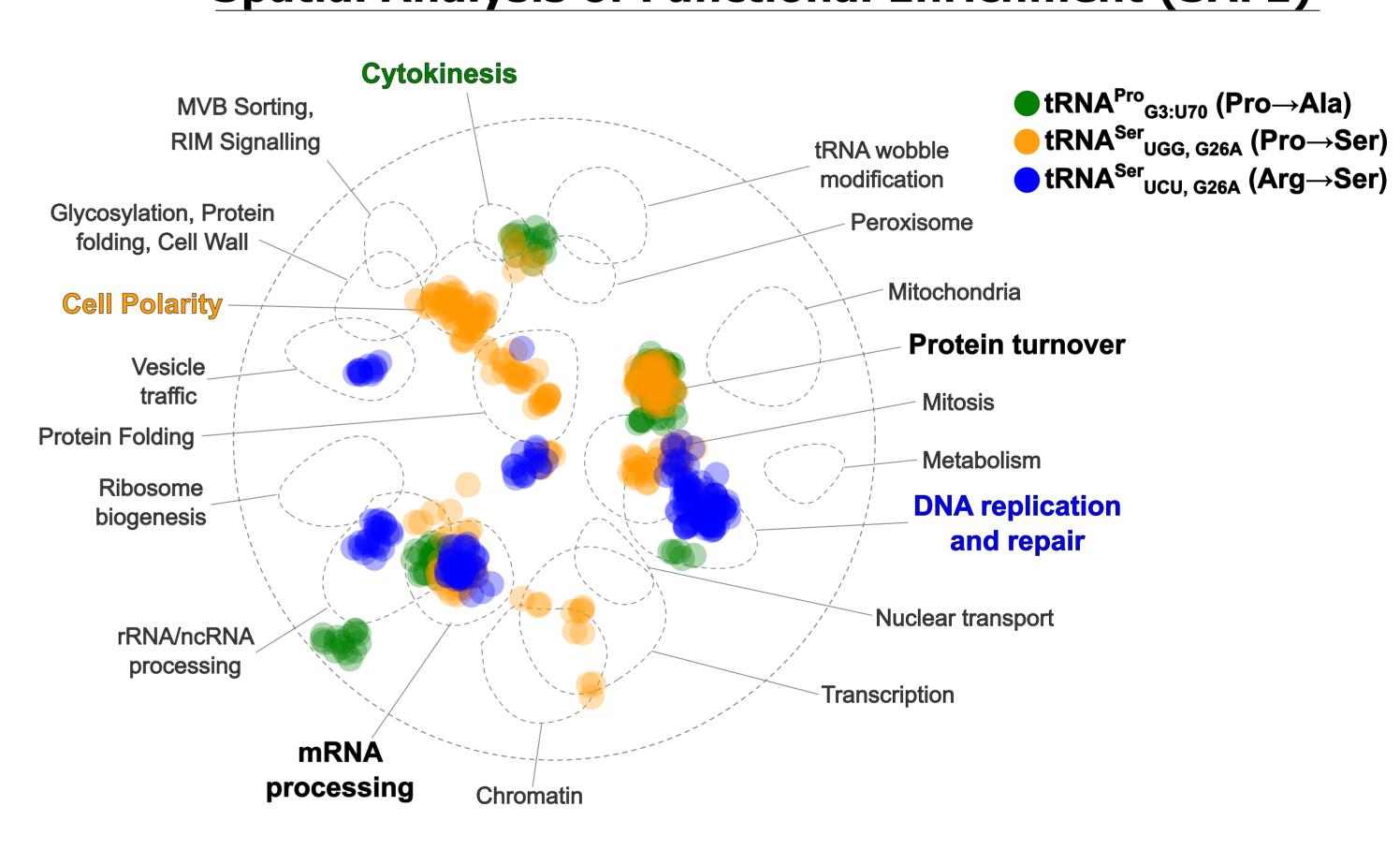
• tRNA[Ser] UCU, G26A induced ~3-fold heat shock repsonse, while tRNA[Pro] G3:U70 did not induce a significant heat shock response
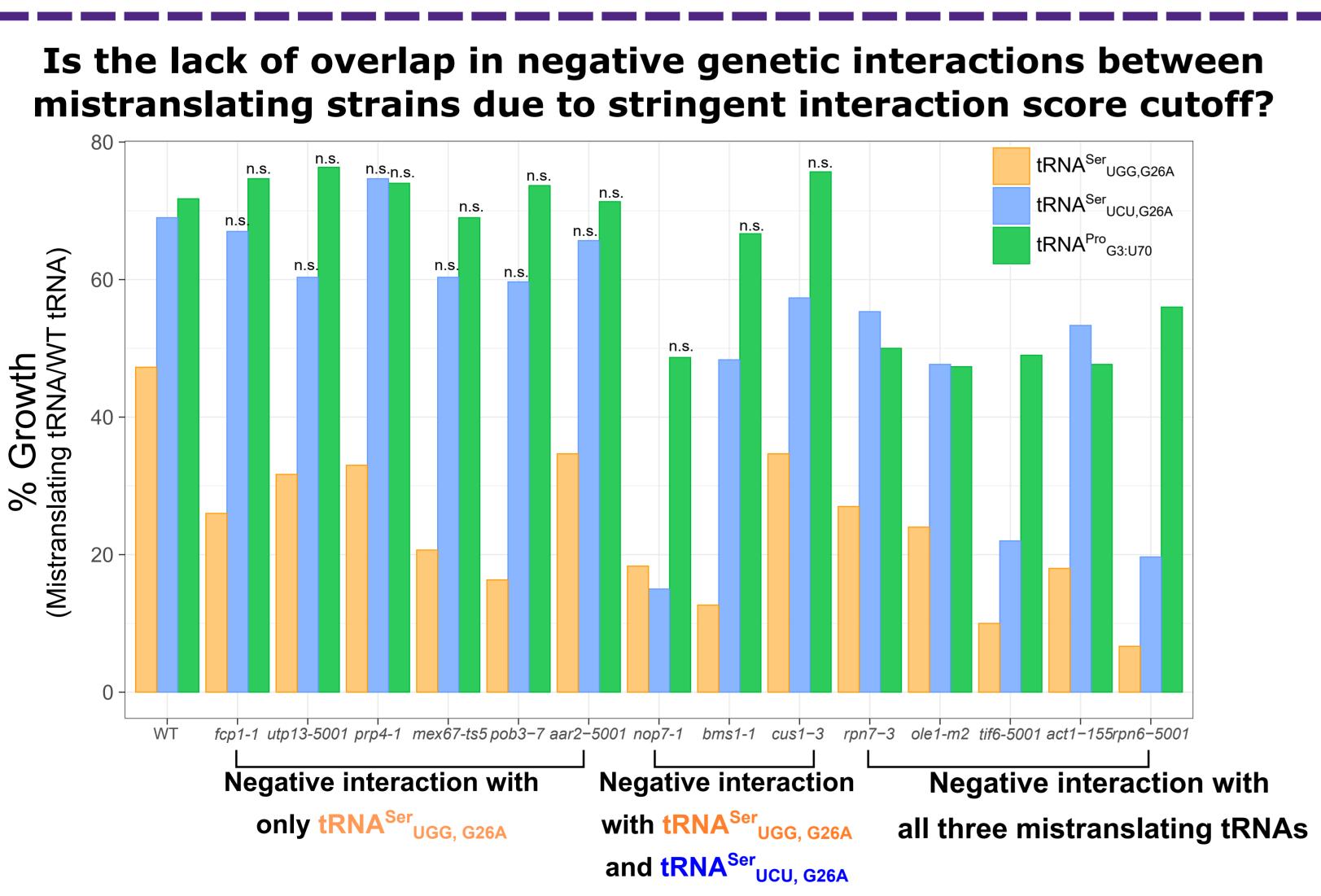
7. Genetic interactions with mistranslating tRNAs

Negative genetic interactions between each mistranslation tRNA and a collection of temperature sensitive alleles were identified through a synthetic genetic array analysis

Spatial Analysis of Functional Enrichment (SAFE)

tRNA[Pro] G3:U70 (Pro→Ala)
tRNA[Ser] UGG, G26A (Pro→Ser)
tRNA[Ser] UCU, G26A (Arg→Ser)

Cytokinesis, MVB Sorting, RIM Signalling, Glycosylation, Protein folding, Cell Wall, Cell Polarity, Vesicle traffic, Protein Folding, Ribosome biogenesis, rRNA/ncRNA processing, mRNA processing, Chromatin, tRNA wobble modification, Peroxisome, Mitochondria, **Protein turnover**, Mitosis, Metabolism, **DNA replication and repair**, Nuclear transport, Transcription

Is the lack of overlap in negative genetic interactions between mistranslating strains due to stringent interaction score cutoff?

% Growth (Mistranslating tRNA/WT tRNA)

tRNA[Ser] UGG,G26A
tRNA[Ser] UCU,G26A
tRNA[Pro] G3:U70

Negative interaction with only tRNA[Ser] UGG, G26A

Negative interaction with tRNA[Ser] UGG, G26A and tRNA[Ser] UCU, G26A

Negative interaction with all three mistranslating tRNAs

We randomly selected 14 strains that had negative genetic interactions with tRNA[Ser] UGG, G26A and transformed them with plasmids expressing each of the three mistranslating tRNAs

While there was more overlap, there were unique alleles that were only synthetic with one or two tRNA variants, suggesting different kinds of mistranslation have unique genetic interactions

Does tRNA[Ser] UGG, G26A have more interactions because it mistranslates at higher frequencies?

% Growth (Mistranslating tRNA/WT tRNA)

tRNA[Ser] UGG,G26A
tRNA[Ser] UGG,U33G

We measured the synthetic effect between the same 14 randomly selected alleles and another serine at proline mistranslating tRNA (tRNA[Ser] UGG, U33G) which mistranslates at ~3% compared to ~5% for tRNA[Ser] UGG, G26A)

We found 11/14 of the alleles also had negative genetic interactions with tRNA[Ser] UGG, U33G, suggesting that severity of mistranslation plays a role for some genetic interactions, but most are a result of the type of substitution

8. Conclusions

Conclusions:

• Both frequency of mistranslation and type of substitution impact the effect of mistranslation on cellular phenotypes

• Based on the unique responses observed for different mistranslating tRNAs, we believe that in addition to exacerbating diseases caused by protein mis-folding, naturally occurring mistranslating tRNA variants have the potential to negative influence specific diseases

Contact me if you have any questions:
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References and Acknowledgments

References
1. Hoffman et al. (2017) Genetic selection for mistranslation resues a defective co-chaperone in yeast. Nucleic Acids Res. 45(6): 3407-3421.
2. Berg et al. (2019) Modulating mistranslation potential of tRNASer in Saccharomyces cerevisiae. GENETICS. 213(3): 849-863.
3. Berg et al. (2019) Targeted sequencing reveals expanded genetic diversity of human transfer RNAs. RNA Biol. 16(11): 1574-1585
4. Tong et al. (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294: 2364-2368.

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