tRNA Revolution: Advances in tRNA-Based Therapies

Thaanya Amarasekara, Olivia Roumaya, Grace Johnecheck, and Brandon Iwaniec
Members of Jane Jackman's Lab, Chemistry and Biochemistry Department

CENTER FOR RNA BIOLOGY OHio

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

What is tRNA?

Transfer RNAs (tRNA) act as an intermediate molecule in cellular protein synthesis process. tRNA molecules are responsible for interpreting the information contained in the sequence of messenger RNA (mRNA) to accurately translate the genetic information stored in DNA.

2D and 3D structures of tRNA

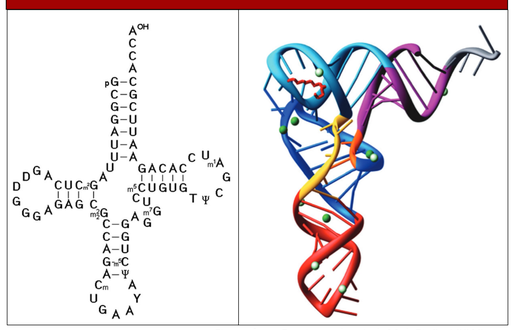

Figure from Barciszewska et al. *Mol. BioSyst* 2016.

- tRNA is made of four nucleotide bases (A, C, G, U) and modified bases.
- tRNA forms a clover-leaf structure due to base pairing between A-U and G-C.
- This clover-leaf structure folds into an L-shaped 3-dimensional structure.

Role of tRNA in the cell

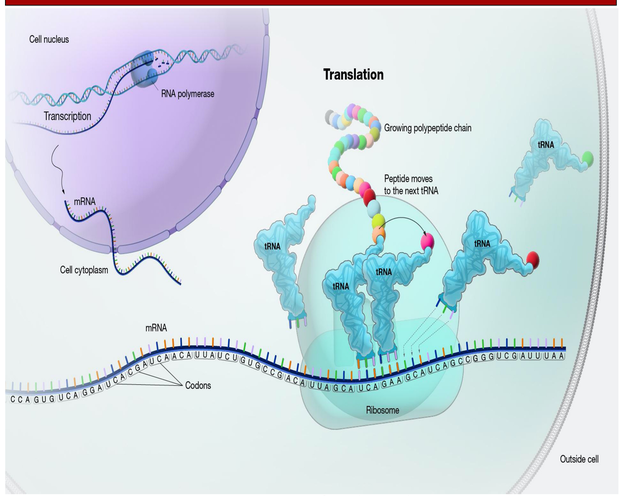

Figure from Clancy and Brown, *Nature Education*, 2008.

- Inside the ribosome, two tRNAs, one with the growing polypeptide chain and the other with a new amino acid, come together.
- The ribosome facilitates the formation of a peptide bond between the amino acid on the incoming tRNA and the growing polypeptide chain on the other tRNA.

tRNA interprets the genetic code

First letter of codon (5' end)

Second letter of codon

	U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Figure 27-7
Lehninger Principles of Biochemistry, Sixth Edition

- Genetic code is made up of three-letter sets of nucleotides (codons), representing 1 of the 20 different amino acids.
- Each amino acid codon usually has multiple tRNAs partners.

A single tRNA therapeutic can target many different diseases

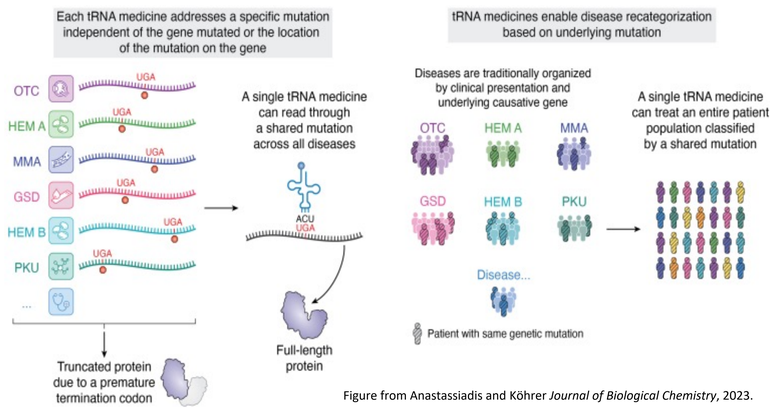

Figure from Anastassiadis and Köhrer *Journal of Biological Chemistry*, 2023.

- Some diseases are caused by a single mutation that may overlap with other diseases, e.g., two different diseases in two different patients from the same mutation.
- tRNA therapeutics would make it possible to target only that mutation, effectively treating anyone with that single mutation, no matter the disease.

Three types of tRNA medicine to address three different types of mutations

Disease-associated mutation	tRNA medicine	Mechanism of action
Single nucleotide substitution → Premature termination codon (PTC) Wild type (WT) -CAG-CUG-CGA-AGC-GAC-CGA- Mutant -CAG-CUG-UGA-AGC-GAC-CGA- PTC Truncated protein	tRNA engineered to recognize PTC* Amino acid found at PTC in WT protein tRNA anticodon recognizes PTC	Therapeutic tRNA decodes PTC → Full-length WT protein synthesis Mutant -CAG-CUG-UGA-AGC-GAC-CGA- Full-length WT protein
Nucleotide(s) insertion or deletion → Frameshift (FS) WT -CAG-CUG-CGA-AGC-GAU-AGA- +1 reading frame Mutant -CAG-GCU-GCG-AAG-CGA-UAG-A +1-nt insertion PTC Scrambled and truncated protein	tRNA engineered to recognize 4-nt codon* tRNA anticodon recognizes FS	Therapeutic tRNA decodes FS to restore reading frame → Full-length protein synthesis Restored reading frame Mutant -CAG-GCUG-CGA-AGC-GAU-AGA- Full-length WT protein
Single nucleotide substitution → Missense mutation (MS) WT -CAG-CUG-CGA-AGC-GAC-CGA- Mutant -CAG-CUG-AGA-AGC-GAC-CGA- Mutant codon Mutated protein	tRNA engineered to recognize MS codon* Amino acid found at MS tRNA anticodon recognizes MS	Therapeutic tRNA recodes MS codon → WT protein synthesis Mutant -CAG-CUG-AGA-AGC-GAC-CGA- WT protein

* Specificity for the target codon should be designed into tRNA medicine

Figure from Anastassiadis and Köhrer, *Journal of Biological Chemistry*, 2023.

tRNA pool therapeutics

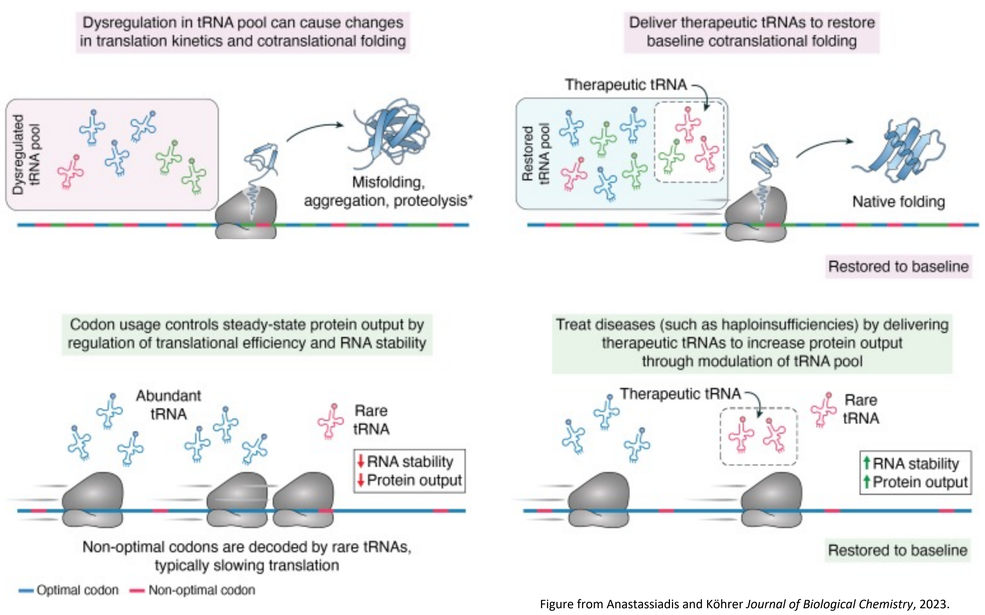

Figure from Anastassiadis and Köhrer *Journal of Biological Chemistry*, 2023.

- tRNAs vary in supply and demand based on the codons required.
- If there are too few of a rare tRNA, translation will be slower, which can cause decreased protein output and misfolded proteins.
- tRNA pool therapeutics adds in the rarer tRNAs to increase translation speed.

tRNA medicine design workflow

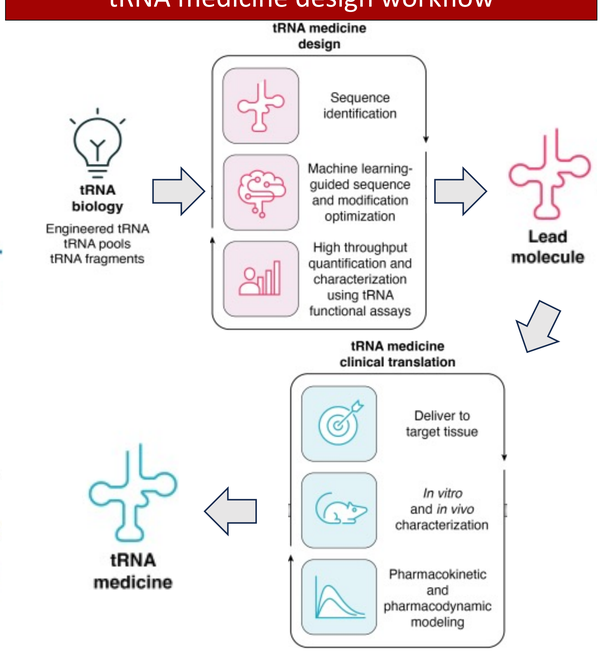

Figure from Anastassiadis and Köhrer *Journal of Biological Chemistry*, 2023.

Summary

- tRNA-based medicines are under development
- tRNA therapeutics offer hope to patients who are suffering from rare and ultra -rare diseases.