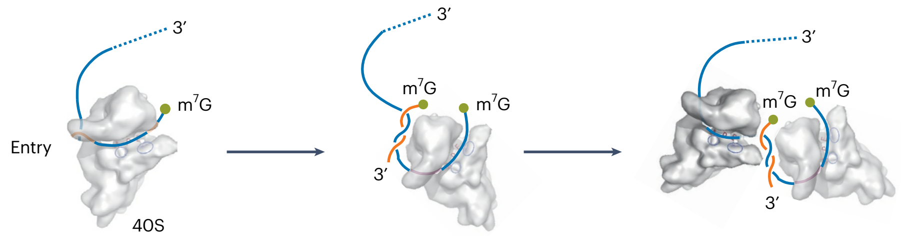
Molecular engineering

https://doi.org/10.1038/s41592-025-02998-4

Trans-RNAs to program translation initiation



The relative orientation of two ribosomes recruited from the original cap (light gray) and the *trans*-cap (dark gray); adapted from L. Jia et al. *Nat. Biotechnol*. https://doi.org/10.1038/s41587-025-02897-1 (2025), Springer Nature America, Inc.

Eukaryotic mRNAs can have multiple translation initiation sites, including non-canonical, non-AUG codons. Start codon selection is a crucial step in mRNA translation and is influenced by both *cis*-regulatory elements and *trans*-acting factors. This dynamic regulation of alternative translation is crucial for cellular homeostasis, and its dysregulation is linked to various human diseases. Very few tools are currently available to modulate start codon selection on individual mRNAs. Co-corresponding authors Yaser Hashem from Université de Bordeaux and Shu-Bing Qian from Cornell University, along with colleagues, have developed a *trans*-RNA system that directs start codon selection on individual mRNAs without altering the original sequence, which offers a new tool to modulate translation initiation.

Translation of upstream open reading frames (uORFs) in mRNAs with multiple start codons can suppress downstream initiation sites. The researchers designed antisense RNA oligonucleotides (oligos) with a functional 5′ cap to bypass uORFs, which enabled ribosomes to capture downstream start codons. However, this strategy presents a caveat: although the ribosome recruited by the capped RNA oligo can bypass the double-stranded RNA (dsRNA) bridge and reach the target mRNA to initiate translation at downstream start codons, its unwinding of the dsRNA bridge ultimately causes the RNA oligo to dissociate.

To address this caveat, the authors introduced a short uORF into a green fluorescence protein (GFP) reporter that encodes a short tracer peptide, and then used a monoclonal antibody to measure the translation in HEK293 cells that stably express H-2K^b (HEK293-K^b cells). The results showed that only a small fraction of scanning ribosomes skipped the structured uORF, which indicates the potential of *trans*-RNAs to influence start codon selection. Additionally, they placed dsRNA near the ribosome's exit site, which confirmed that a secondary structure near the 5′ end of mRNA is more resistant to unwinding.

Qian notes that the most challenging aspect for the team was determining how ribosomes are loaded through a capped antisense RNA. "This pathway resembles the conventional 5′ cap-mediated initiation mechanism, but it is not identical, and uncovering the distinguishing features required significant effort and careful experimentation," he says. The researchers synthesized *trans*-RNAs that were complementary to regions between the uORF and GFP, with varied 5′ unpaired extensions. Transfection into HEK293-K^b cells showed that fully paired *trans*-RNAs significantly increased GFP levels as compared with control *trans*-RNA with random sequences. Enhanced GFP translation and, to a lesser extent, an increase in uORF translation suggested that *trans*-RNAs promote ribosome scanning and downstream translation of GFP and the uORF. A real-time fluorescence duplex unwinding assay supported these findings, by showing increased ribosome loading from the original cap and enhanced leaky scanning. The study also demonstrated that *trans*-RNAs function independently of the original cap, as removing the 5′ end cap from *trans*-RNAs eliminated their activity in target mRNA translation.

The authors conclude that *trans*-RNAs modulate translation initiation by directing ribosome loading and scanning. The *trans*-RNA system provides a shortcut for ribosomes through the *trans*-cap, positively regulating canonical translation through a looping mechanism. This system allows ribosomes to favor start codons near the *trans*-cap, and enhances translation efficiency.

With these exciting results, the authors explored programmable translation initiation of the endogenous mRNAs *Atf4* and *Cebpb* by *trans*-RNAs. They also systematically identified endogenous *trans*-RNAs that control alternative translation initiation using global translation initiation sequencing (GTI-seq) datasets obtained from HEK293 cells. In the future, Qian plans "to develop more-sensitive sequencing methods to identify these natural *trans*-RNAs across different tissues and under various stress conditions." In the long term, he hopes that engineered *trans*-RNAs will ultimately become broadly applicable tools – much like today's microRNAs and antisense oligos – especially once modifications are incorporated to enhance their stability and potency.

Aparna Anantharaman
Nature Methods

Original reference: *Nat. Biotechnol*. https://doi.org/10.1038/s41587-025-02897-1 (2025).