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RNA-based translation activators for targeted gene upregulation

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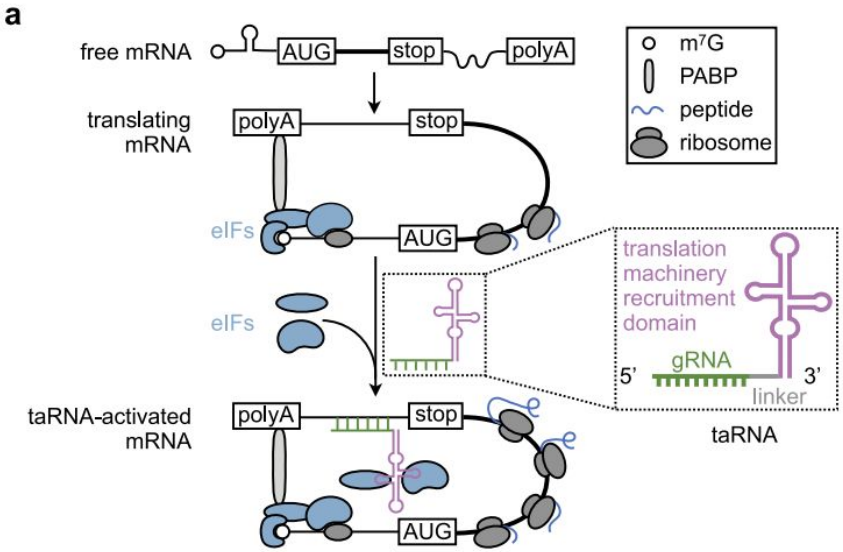
Background

- The regulation of gene expression is fundamental to cell function and is controlled at multiple levels—including transcription, mRNA processing, mRNA stability, and translation.

- This paper introduces RNA-based technology called taRNAs (translation activation RNA) that enhance gene expression

Key Features of taRNA



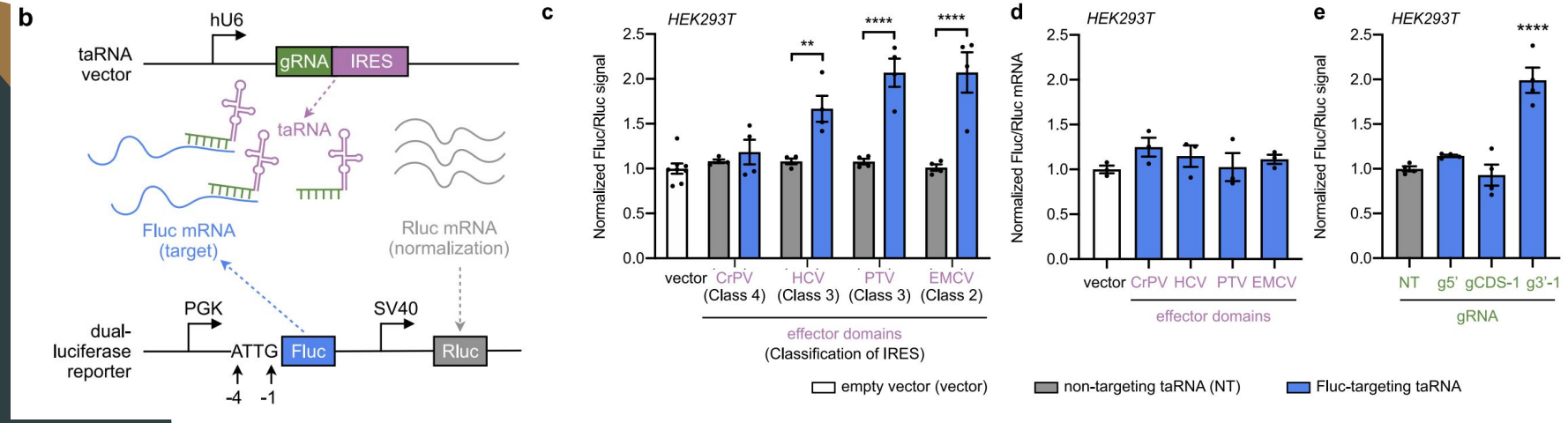
- A guide RNA (gRNA) that binds a specific target mRNA.
- A short linker (5nt)
- An effector domain derived from an internal ribosome entry site (IRES) that recruits translation initiation factors (like eIF3 or eIF4G).

Aim and Objective

1. Show that taRNA are capable of translational enhancement
2. Test a variety of IRES-derived effector domains for their ability to recruit translation machinery.
3. Identify optimal guide RNA sequences
4. Validate taRNAs in multiple biological contexts, including:
    a. Cell lines (HEK293T, N2a, MDA-MB-231),
    b. Primary rat neurons,
    c. In vivo mouse liver models,
    d. And importantly, iPSC-derived neurons from a SYNGAP1 haploinsufficient patient.

Initial screening of taRNAs for translational enhancement

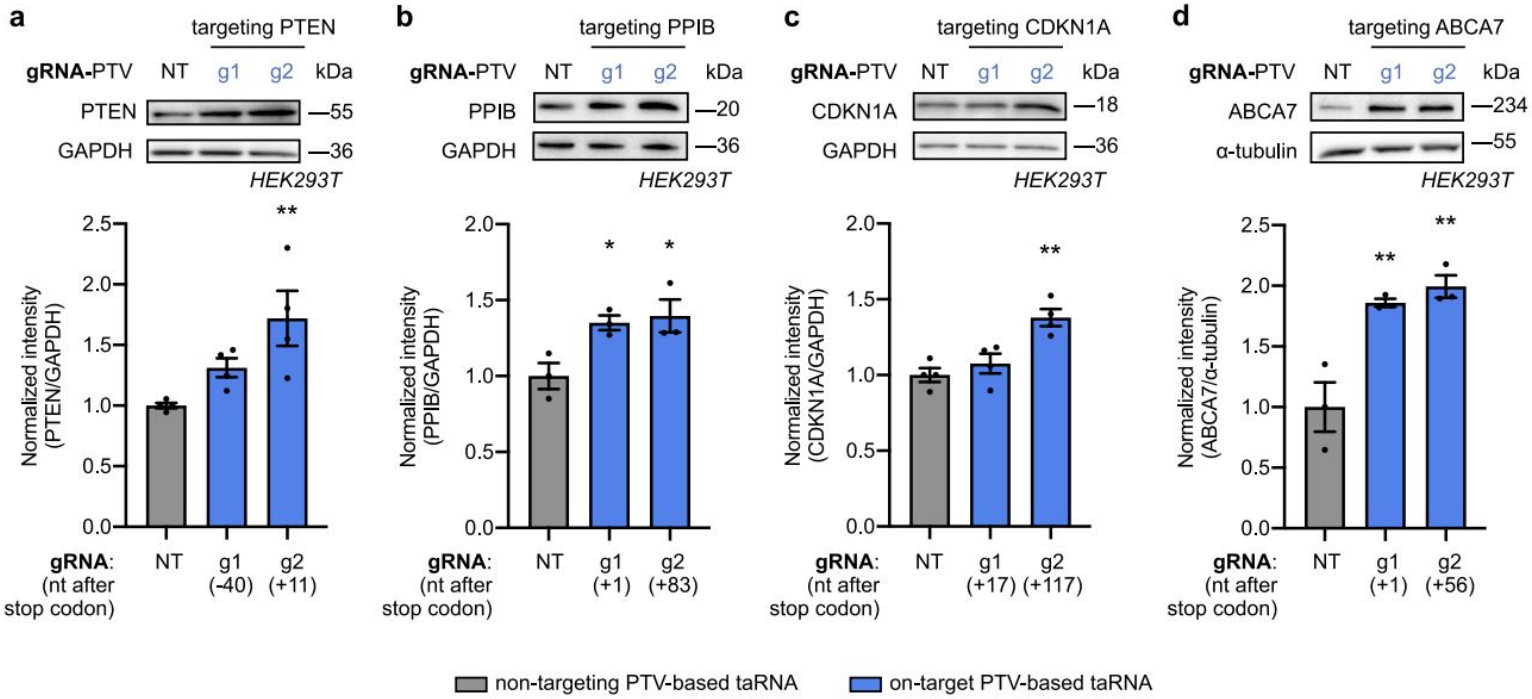
- Researchers used a dual-luciferase reporter system
- Demonstrated that these taRNAs could enhance translation from exogenous mRNAs in a reporter system



Optimization of taRNA gRNA position

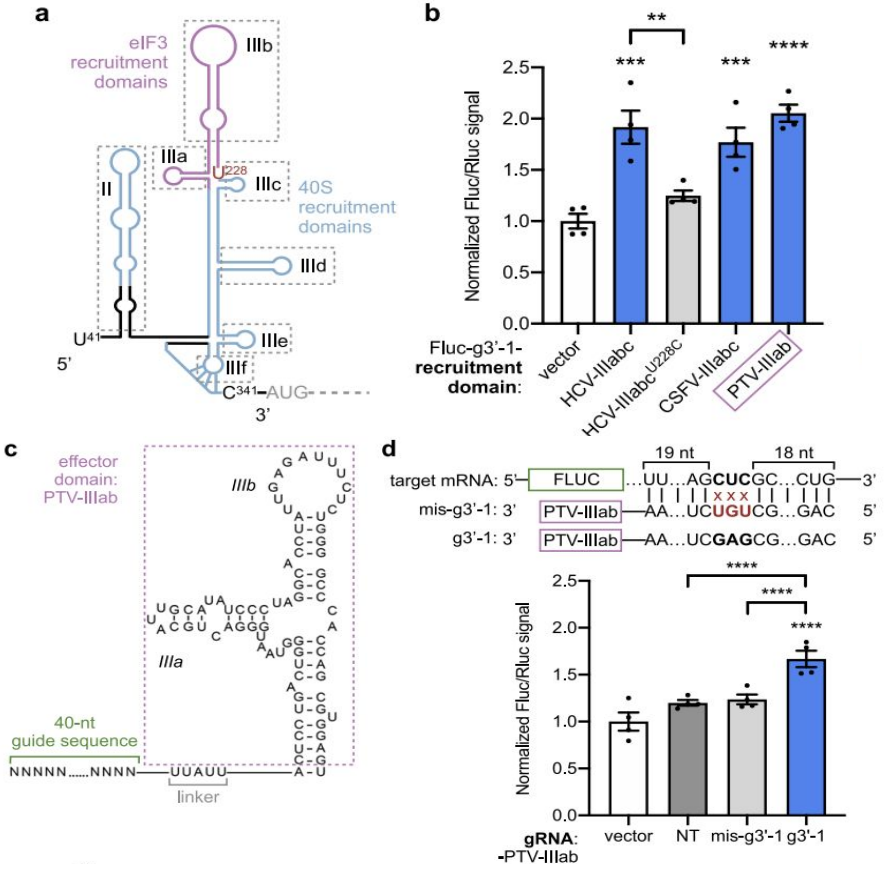
- Target 3′ UTR
- **RNAfold**: Used to avoid gRNA sequences with strong internal secondary structures.
- **RNAup**: Used to estimate the accessibility of the target mRNA region.
  BLAT: Used to avoid potential off-target binding to other transcripts.
- **Transcription considerations**: Avoid sequences resembling RNA Pol III terminators (e.g., stretches of uridines) that could cause premature transcription termination.

taRNA enhances translation of endogenous mRNA



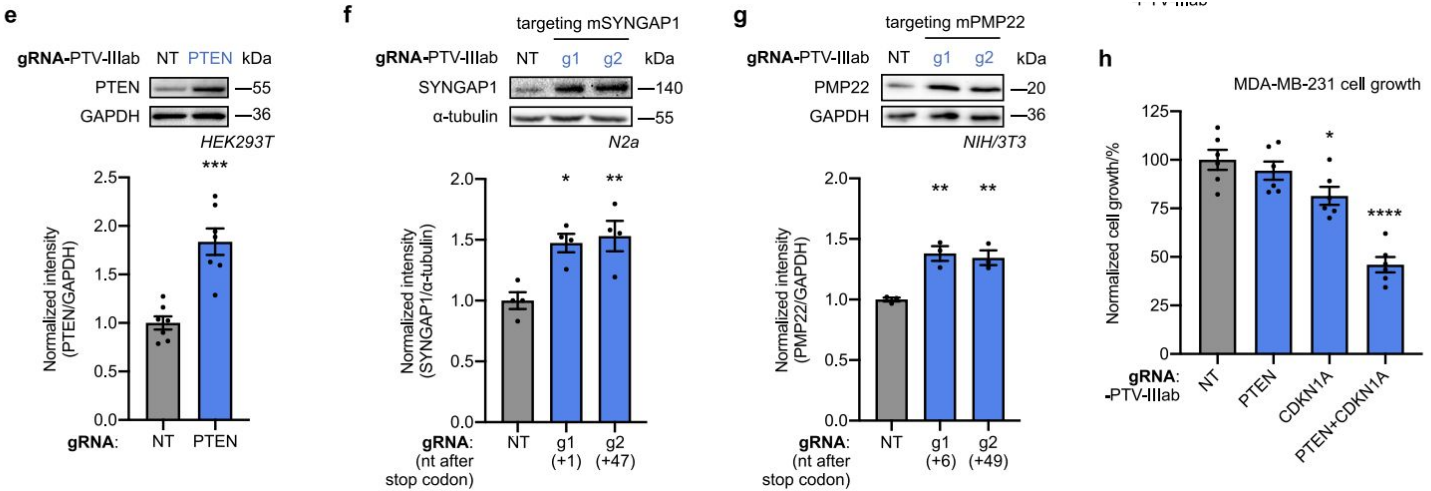
taRNA can be miniaturized using truncated domains

a. Diagram of complete IRES structure of HCV
b. Attached conserved domains of different IRES to taRNA and tested via dual-luciferase assay
c. Schematic of taRNA PTV-IIIab
d. Test for various gRNA positions

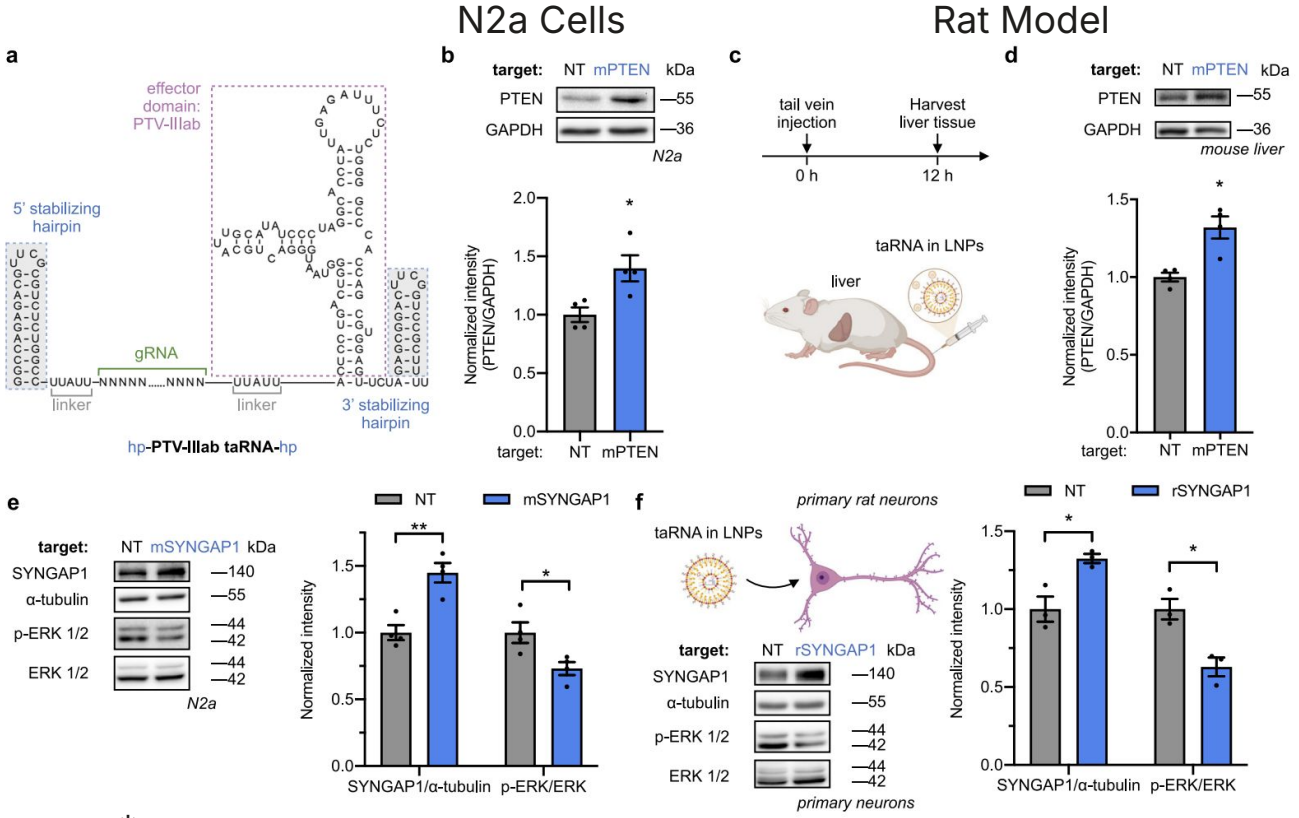


taRNA can be miniaturized using truncated domains

- After determination of optimal truncated IRES (PTV-IIab), taRNA was transfected into different cell models and targeted various disease related mRNA



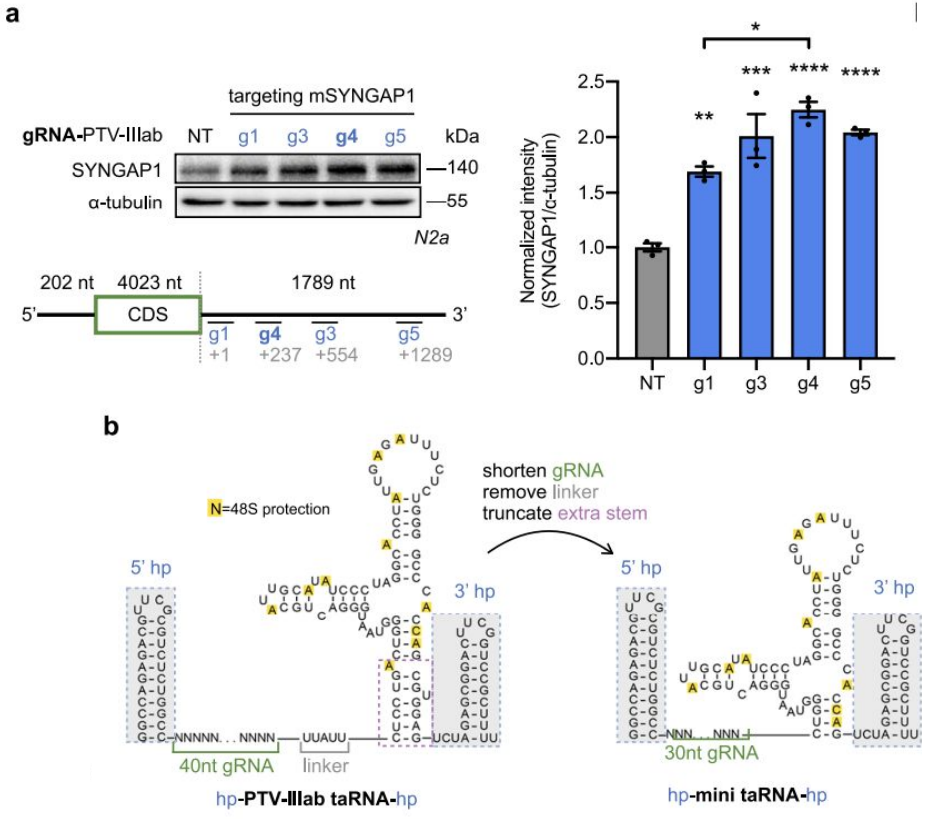
taRNA can functionally upregulate proteins in cell and animal models



*SYNGAP1 negatively regulates ERK signaling.

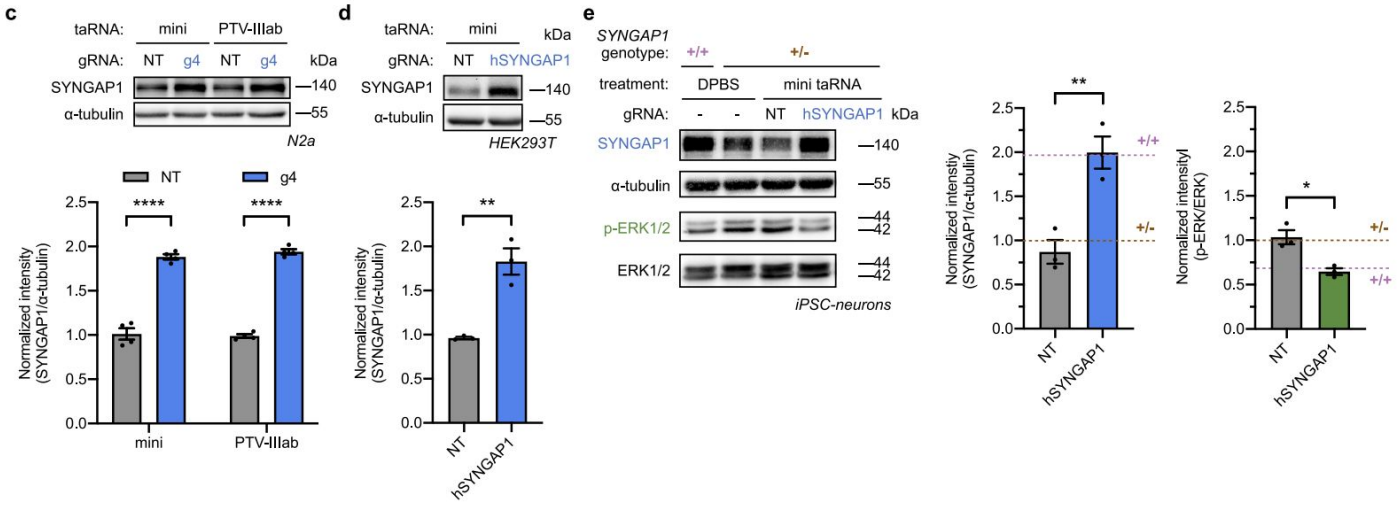
taRNA can rescue protein expression and function in SYNGAP1 haploinsufficient neurons

a. Screened multiple SYNGAP1-targeting gRNAs in 3′UTR
b. Constructed ta RNA with shorter gRNA and truncated IRES domain



taRNA can rescue protein expression and function in SYNGAP1 haploinsufficient neurons

c. Compared full-size vs mini taRNA
d. Tested mini taRNA on human SYNGAP1 in HEK293T
e. Delivered mini taRNA to SYNGAP1+/– iPSC-derived neurons and Restored SYNGAP1 protein & rescued ERK signaling



Summary

- taRNAs are small, modular RNAs that enhance translation of target mRNAs by recruiting eukaryotic initiation factors (eIFs).
- They act post-transcriptionally, without altering mRNA levels or requiring genome editing.
- Function similar to PPR Proteins, however employ gRNA and IRES (RNA based effector domains) to increase translation.
- Can be employed in various cell and animal models.