Molecular Therapy
Nucleic Acids
Commentary

SINEUPs to boost translation

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https://doi.org/10.1016/j.omtn.2023.06.003

The genome is broadly transcribed by RNA polymerase II, and most of the transcripts are non-coding RNAs.[1] Long non-coding RNAs (lncRNAs) exert their regulatory role by interacting with proteins to activate their function, or with protein complexes by acting as a scaffold, but also with DNA or RNA by complementary sequence pairing. A large fraction of genomic regions is transcribed from DNA filaments generating both sense and antisense RNA molecules, which can form double-stranded RNAs. The interaction of a non-coding RNA with an mRNA might regulate the expression of the coding gene by different mechanisms, including the modulation of mRNA splicing or activation of silencing. Interestingly, antisense lncRNAs can also stimulate mRNA translation, which requires two functional domains: a complementary region for the 5′ UTR of the mRNA and a ribosome-recruiting domain. These antisense lncRNAs were named SINEUPs as they contain an inverted SINEB2 element and UP-regulate translation.[2] SINEUPs are very interesting RNAs. They are, in all respects, *trans*-acting translation factors. Importantly, SINEUPs increase translation of their mRNA targets by only 1.5- to 3-fold. This might seem negligible, but it is within physiological range, allowing for the consideration of SINEUPs for RNA-based therapies for pathologies caused by haploinsufficiency. In the previous issue of *Molecular Therapies – Nucleic Acids*, Pierattini et al. further studied the RNA requirements of SINEUPs to better modulate their translation efficiency.[3] In particular, they analyzed the role of m6A modification. Cellular RNAs contain a number of modified residues that influence their function. Among these, N6-methyladenosine (m6A) is the most abundant modification of both coding and non-coding RNAs. The presence of m6A on RNAs has been shown to control stability, splicing, and translation efficiency.[4]

In this study, Pierattini et al. first demonstrated the presence of m6A deposited by METTL3 on the SINEUPs analyzed and mapped the m6A next to the DRACH consensus motif (D = G, A, or U; R = G or A; H = C, A, or U) within the inverted SINEB2 element. To understand SINEUP's function, it is worth noting that the inverted SINEB2 region within SINEUP folds into a stem-loop structure, which resembles an internal ribosome entry site (IRES). By knocking down METTL3 or generating SINEUP mutants on putative m6A sites, the authors demonstrated that this modification is required to enhance translation efficiency. SINEUPs increase translation efficiency by promoting the assembly of the ribosome on its AUG target site, leading to a significant increase in polysomes on the mRNA.

The translation initiation of most eukaryotic mRNAs depends on the CAP structure at the mRNA 5′ end that is recognized by eukaryotic initiation factor 4E (eIF4E), a subunit of the eIF4F complex. The eIF4F bound by CAP then recruits the 40S ribosomal subunit, which when associates with translation initiation factors, and translocates on the 5′ UTR to reach the first AUG within the Kozak consensus sequence. Nevertheless, RNAs can be translated even in the absence of CAP. Indeed, the translation of uncapped mRNAs and even circular RNAs occurs in a CAP-independent manner. CAP-independent translation of cellular mRNAs depends on m6A modifications within the 5′ UTR, which recruits eukaryotic initiation factor 3 (eIF3) to initiate translation in the absence of eIF4E and involves 5′ end mRNA scanning.[5] IRES-dependent translation of mRNAs[6] or circular RNAs, which cannot rely on CAP, also depends on m6A and has demonstrated that this modification is required to bind the YTH domain protein YTHDF3.[7]

Instead of initiating translation of uncapped RNAs or circular RNAs, SINEUPs enhance the translation of a capped mRNA, which is already translational competent. SINEUP, thanks to its complementary region, binds to its target mRNA, adding an m6A-modified IRES-like structure to the mRNA (Figure 1). Upon this binding, the translation of a specific mRNA is activated by two independent mechanisms, which would give rise to a number of different scenarios. Are these different initiation mechanisms acting on different mRNAs or on the same mRNA molecule? In this latter case, the mRNA 5′

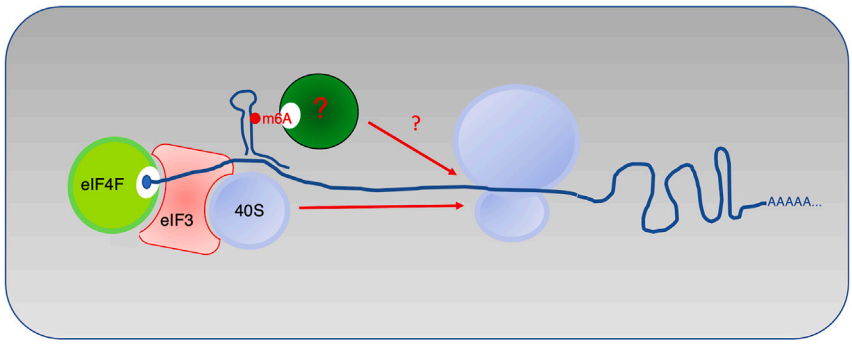

Figure 1. Capped mRNA also binding a SINEUP

The CAP structure binds the initiation factor eIF4F complex, interacting with eIF3 to recruit the 40S subunit that translocates to reach the AUG within the Kozak consensus sequence. Above, the SINEUP carrying an m6A modification on the stem-loop IRES-like structure binding the same mRNA also binds a currently unidentified factor (possibly a YTH or eIF3) to increase translation efficiency.

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UTR would be quite crowded, implying that the translation initiations must be highly dynamic (Figure 1). The limited number of mRNA targets, although boosted by two independent translation initiation mechanisms, would still be limited by the number of mRNAs, resulting in a relatively modest increase in translation efficiency.

Interestingly, from the results of this work, we can also predict that any open reading frame (ORF) present on cellular RNAs, either circular or linear, could be translated. Upstream of an ORF, it would be sufficient to carry a sequence recognized by the antisense portion of a SINEUP expressed in the same cell. This mechanism, if confirmed, would increase the number of proteins that a cell can produce. Natural SINEUPs play a regulatory role that is opposite to microRNA's function and, if expressed in significant numbers, would widen the repertoire of translational regulation. A more systematic search of natural SINEUPs could clarify the relevance of this regulation and identify the binder to the m6A modification, which could be a YTH protein or even eIF3. Another still unresolved and intriguing point is the early detachment of SINEUP, as the authors did not find it on the polysome. Further experiments are required to clarify these aspects.

To compensate for the loss of gene function, gene therapy often relies on the overexpression of therapeutic genes. However, in a number of neurodegenerative diseases the problem is due to haploinsufficiency. This work introduces the possibility of using synthetic SINEUPs as a strategy to increase translation efficiency in patients with pathologies due to haploinsufficiency. In these cases, a relatively modest increase in mRNA translation of the healthy allele of 2- to 3-fold is ideal to obtain nearly physiological levels of expression of the candidate gene.

ACKNOWLEDGMENTS

This work was supported by a grant from CN3 - National Center for Gene Therapy and Drugs based on RNA Technology.

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