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TRANSLATION

Start upstream to relieve stress

Many mammalian mRNAs contain short, upstream ORFs (uORFs) in their 5′ untranslated regions (5′ UTRs), some of which are initiated at non-AUG start codons. The extent of translation of uORFs and other short ORFs and its implications remain unclear. Starck *et al.* developed a technique they named tracing translation by T cells (3T), to systematically measure translation outside annotated coding sequences. They found that translation of uORFs during the integrated stress response (ISR; induced by stimuli such as heat stress, misfolded proteins, viral infection or amino-acid deprivation) could be required for the translation of their downstream ORFs.

In 3T the translation of RNA elements is monitored by introducing sequences encoding tracer peptides that, if translated, are loaded onto major histocompatibility complex class I (MHC I) molecules. These molecules are displayed on the cell surface, where they are recognized by a peptide–MHC I-cognate T cell hybridoma expressing a reporter gene, which is expressed only following the interaction with tracer peptide-loaded MHC I molecules.

The authors used 3T to explore how the translation of stress-response mRNAs — many of which have several uORFs — is resistant to the global translation shutdown that occurs during the ISR. The shutdown is mediated by phosphorylation of the α-subunit of eukaryotic initiation factor 2α (eIF2α) and the concomitant downregulation of translation initiation at AUG start codons.

The mRNA encoding the stress-response chaperone immunoglobulin heavy chain-binding protein (BiP; also known as GRP78) has two (putative) uORFs: one initiated at the Leu UUG codon at position −190 (relative to the BiP coding sequence) and another at the Leu CUG codon at position −61. The translation of both was confirmed by 3T and did not change following eIF2α phosphorylation. The expression of the alternative initiation factor eIF2A was induced by the ISR, and its depletion reduced the translation of the −190 UUG uORF (but not translation globally) and, importantly, the expression of BiP during the ISR. Deletion of the −190 UUG uORF in combination with eIF2A depletion had a synergistic effect.

In summary, eIF2A and two uORFs maintain translation of the BiP coding sequence during ISR-induced translation shutdown, possibly to replace the inhibited eIF2α-dependent translation. The sensitivity of the 3T method should enable the detection and annotation of so far unknown, small ORF-encoded peptides.

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ORIGINAL ARTICLE Starck, S. R. *et al.* Translation from the 5′ untranslated region shapes the integrated stress response. *Science* http://dx.doi.org/10.1126/science.aad3867 (2016)

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