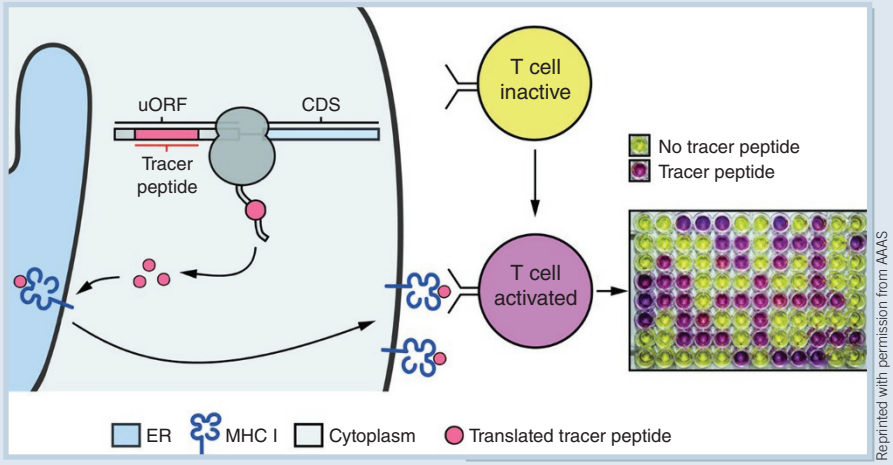
Using T cells to detect noncanonical translation



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A wealth of recent research has revealed that much more of the eukaryotic genome is transcribed than previously thought, although it is still unclear how much of this transcription is functional and how much is spurious. At the same time, it is becoming apparent that more of the transcriptome is translated than previously anticipated. Again, the question arises as to how much of this translation is functional. Recent ribosome profiling studies have suggested the widespread translation of short open reading frames (sORFs), some of which initiate with non-AUG start codons. However, ribosome occupancy does not necessarily equate to translation, and difficulties in detecting small peptides, which may be rare and/or unstable, make it unclear how many of these sORFs give rise to peptide products. Detection of such products is important to address whether their translation or the peptides themselves have biological functions. To tackle these questions, *Starck et al.* (Science doi:10.1126/science.aad3867, 29 January 2016) have developed a method called 'tracing translation by T cells' (3T) to monitor translation of sORFs.

The 3T method (pictured) exploits the sensitivity and specificity of T cells to detect translation products from sORFs. Nearly all vertebrate cells present a mixture of peptides on their surface via major histocompatibility complex class I (MHC I) molecules. These peptides are generated by proteasomal degradation of proteins in the cytoplasm and therefore represent the intracellular protein content. Each peptide is loaded onto an MHC I molecule in the endoplasmic reticulum (ER), and the complex is then transported to the cell surface. Any nonself peptide can be recognized by a T cell bearing a T-cell receptor specific for that antigen. Peptides from conventional translation events are displayed as well as those from noncanonical translation events, such as from 5′ or 3′ untranslated regions (UTRs), alternate reading frames and non-AUG start codons. In the 3T method, cells are transfected with DNA or mRNA vectors encoding a tracer peptide within a sequence, such as a 5′ UTR, that is suspected of being translated. If a tracer peptide is produced, it will be loaded onto MHC I molecules and displayed on the cell surface. The peptide can be detected by a T cell specific for that antigen; moreover, the T cell is engineered to express β-galactosidase upon recognition of the peptide–MHC I complex, thus allowing the use of a colorimetric assay to quantitatively monitor the T-cell response.

The authors have used 3T to study sORFs upstream of mRNAs encoding stress-response genes. Stress stimuli have long been known to alter translation. The integrated stress response (ISR) activates kinases that phosphorylate the α subunit of eukaryotic initiation factor 2 (eIF2α). This phosphorylation leads to downregulation of eIF2-dependent translation from AUG start codons, thereby conserving resources and promoting cell survival during stress. However, mRNAs encoding stress-response factors escape this general downregulation. It is not clear exactly how those transcripts do so, but features of their 5′ UTRs, including internal ribosome entry sites, nucleotide modifications and upstream ORFs (uORFs) appear to be involved. Starck *et al.* set out to investigate translation of uORFs during the ISR and their role in escape from translational downregulation.

The authors focused on binding immunoglobulin protein (BiP), an essential HSP70-type chaperone in the ER, whose mRNA exhibits high ribosome occupancy in its 5′ UTR. By nesting a tracer peptide in a predicted uORF starting with a UUG (leucine) codon, they have shown that this uORF is translated and that translation persists after stress-induced eIF2α phosphorylation. They also observed that knockdown of eIF2A, an alternative initiation factor known to coordinate noncanonical leucine initiation, impairs translation of the UUG uORF and, importantly, also compromises BiP expression under stress conditions. Therefore, uORF translation appears to be required for persistent BiP translation during stress, although it remains unclear how this process protects the BiP mRNA from translational downregulation.

An intriguing question is whether peptides produced from such noncanonical translation events are important for immune surveillance during cellular stress. Interestingly, the peptide translated from the UUG uORF of BiP, as well as several putative peptides from other non-AUG uORFs in the 5′ UTRs of chaperones, are predicted to have high affinity for human MHC I proteins. Future work will determine the roles, if any, of these peptides in adaptive immune responses.

In summary, the 3T assay has great potential for the study of noncanonical translation. It can be used in any cell type in which the MHC I peptide-presentation pathway is constitutively active, and it can detect very short or unstable peptides. It is also highly sensitive, because T cells are able to detect even a few copies of the peptide–MHC I complex. In combination with ribosome profiling, mass spectrometry and computational approaches, 3T will provide important new insights into the complexity of the proteome and help to uncover new functions of unconventional translation.

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