What is ribosome stalling?

To produce a protein, mRNA must undergo translation, a process where a ribosome moves along the mRNA and reads codons that code for specific amino acids. During this process, paired tRNAs deliver amino acids to the growing polypeptide chain. The ribosome then translocates to the next codon, repeating this process until it reaches a codon that stops translation. The resulting polypeptide chain becomes the protein.

However, sometimes features of the mRNA or conditions in the cell interrupt translation, forcing the ribosome to pause or prematurely stop moving on the mRNA. This is called ribosome stalling.

Browser tracks of the PCSK9 gene, either treated with the drug PF-846 or controls. PF-846 treatment induces ribosome stalls on the first exon while expression levels remain consistent.

What causes ribosome stalling?

Cellular factors and certain mRNA sequences can lead to ribosome stalls, such as RNA secondary structure. If an mRNA has a rigid structure, the ribosome may be unable to smoothly move along the mRNA strand, forcing the ribosome to stall on the strand when it reaches these difficult sections.

Rare codons can also cause ribosome stalling. When a ribosome reads a common codon, there are abundant tRNAs available to quickly deliver the correct amino acid. With rare codons, though, there are fewer tRNAs that carry that amino acid, so the ribosome might wait longer for the tRNA to deliver it. If the ribosome waits too long, it stalls. Relatedly, changing environmental conditions can lead to tRNA depletion and alter stalling patterns during stress responses where certain tRNAs become limiting.

Certain mRNA sequences can cause ribosome stalls as well. For example, repetitive and poly(A) regions of mRNA can be difficult for ribosomes to decode, crowd the mRNA channel, and slow elongation in the peptide exit tunnel. These issues can force the ribosome to stop translation.

How do cells rescue stalls?

When ribosomes stall, it can lead to incomplete, potentially toxic peptides that do not form the correct protein, which can cause diseases. Fortunately, cells have evolved several mechanisms to prevent and rescue stalled ribosomes. For instance, ribosome-associated chaperones bind to ribosomes near the peptide exit tunnel to protect the exposed chain from premature protein folding during ribosome stalls. If the protein is extracted from the ribosome, post-translational chaperones protect and fold the nascent protein or degrade it and the aberrant mRNA if the protein cannot be properly folded. Stalled and collided ribosomal subunits can be split and recycled through ribosome-associated quality control pathways.

However, these mechanisms are not perfect. If an incomplete peptide or aberrant mRNA strand is not destroyed, it can cause cellular stress and contribute to cancer progression and neurodegenerative diseases.

Identifying ribosome stalls

To locate stalls missed by cells, researchers use ribosome profiling. To perform ribosome profiling, researchers lock ribosomes in place and degrade the free mRNA, leaving behind only the fragments of mRNA directly interacting with ribosomes. These fragments are called ribosome protected footprints, and they allow for analyzing translation under specific conditions since they contain only the sequence that was actively being translated.

Once the ribosome footprints are collected, they are mapped to the transcriptome to determine exactly where the ribosomes were translating. If the footprints are accumulating at a specific site and there are very few footprints beyond that spot on the mRNA, then it is likely a ribosome stall site.

Identifying stalls at Eclispebio

At Eclipsebio, we perform ribosome profiling in our eRibo Pro assay. This assay combines ribosome profiling with RNA-Seq, offering nucleotide-level insights into both translation and transcription. By combining the two, researchers can see not only how ribosomes are translating their mRNA but also how transcriptome is expressed.

Interested in finding ribosome stalls on your mRNA? Contact Eclipsebio to learn more.