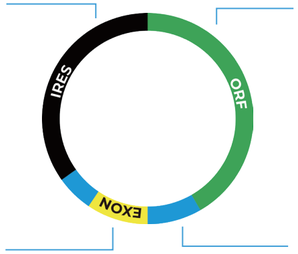
A beginner's guide to circular RNA

Circular RNA (circRNA) is a novel type of single-stranded *in vitro* transcribed (IVT) RNA which forms a covalently closed continuous loop. Unlike conventional linear mRNA, the circRNA structures do not have 5' and 3' ends, which protects them from exonuclease-mediated degradation, and confers enhanced stability. As a result, circRNA have a longer half-life in cells, which can be particularly useful for therapeutic applications where prolonged RNA activity is desired.

What parts make up circRNA and what does each do?

Internal Ribosome Entry Site (IRES):
facilitates cap-independent translation initiation, directly recruits ribosomal subunits, and initiates protein synthesis after the ribosomal subunits are bound

Exon:
the residual sequence of the group I permuted intron-exon (PIE) system. Contains the recognition sequence which helps splice the group I intron to generate the circular target sequence



Open Reading Frame (ORF):
protein coding region of the circRNA

Spacers:
facilitate circularization and provide structural integrity

What applications can circRNA be used in?

- **Protein encoding circRNA as therapeutics and vaccines:**
  enhanced and extended protein expression resulting from circRNA's resistance to degradation may allow for reduced dosing frequency

- **Noncoding circRNAs as therapeutics:**

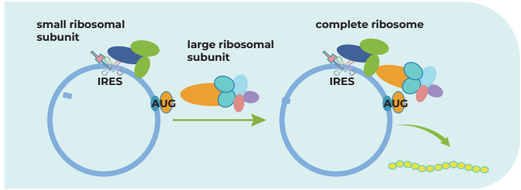
  - **miRNA/protein sponges**- circRNA can sequester miRNA or proteins to regulate gene expression or protein function
  - **siRNAs**- circRNA can be engineered to contain sequences that mimic siRNAs, allowing them to target specific mRNAs for degradation
  - **guide RNAs**- circRNA can be engineered to guide Cas nucleases in CRISPR-Cas gene editing systems by incorporating a targeting sequence to bind to a DNA target and a scaffold sequence that binds to the Cas protein

How are circRNA translated into protein?

The process by which circRNA translates into protein shares many similarities with that of linear mRNA. However, circRNA's unique structure means that the process relies on some different mechanisms as well:

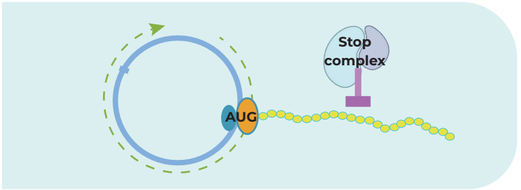
1. Like linear mRNA, for a circRNA to be translated, it must contain an ORF with a start codon (typically AUG).
2. Because circRNAs lack a 5' cap and 3' poly(A) tail, they require an alternative method for ribosome recruitment. This is achieved with an Internal Ribosome Entry Sites (IRES).
3. Once the ribosome is recruited to the circRNA via the IRES, the small ribosomal subunit can scan for a start codon, join with the large ribosomal subunit to form a complete ribosome, and initiate translation (Figure 1).

Figure 1.
IRES-initiated translation of circular RNA



4. The ribosome then translates the circRNA ORF into a protein or peptide in a 5' to 3' direction until it encounters a stop codon. If a stop codon is not recognized, rolling circle translation allows ribosomes to continue translating through multiple rounds, resulting in subsequent copies of the encoded protein (Figure 2).

Figure 2.
Rolling translation of circular RNA



5. Once the ribosome reaches a stop codon, translation is terminated, and the newly synthesized protein is released into the cell to perform its function.

Interested in GenScript's custom IVT circRNA synthesis services? Learn more at
www.genscript.com/ivt-rna-circrna.html