Quantification and Optimization of Circular RNA Stability for Improved RNA Therapeutics

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As mRNA-based therapeutics like COVID-19 vaccines rapidly evolve, degradation remains a key limiter of efficacy and distribution. Circular RNAs (circRNAs) possess a closed-loop structure, conferring enhanced stability and thus a potential solution. This study aims to 1. Quantify stability of circRNA in vitro to identify ideal storage conditions and 2. Improve circRNA stability through optimizations of molecular structure. CircRNAs of length ~1,700 and ~3,350 nucleotides were found to remain stable at room temperature (RT) for 2 weeks and at -20°C and -80°C for 7.5 months. Stability did not improve with storage buffers commonly used for mRNA. CircRNA withstood 10 freeze-thaw cycles without degradation or reduced protein expression. In comparison, mRNA is stable for several hours to a few days at RT and up to 6 months at -80°C, degrading after 5 freeze-thaw cycles. For structural optimization, 14 modified nucleoside triphosphates found to enhance mRNA stability were tested on mNeonGreen circRNA at a 5% substitution rate. Stability and expression were evaluated relative to unmodified circRNA. Stability improved over 16-fold for all optimizations. Expression levels of certain optimizations outperformed the unmodified circRNA 24 hours after digestion, but all performed worse after 96 hours. CircRNA is far more stable than mRNA in a laboratory environment, enabling easier storage and transportation, thus improving accessibility of powerful therapeutics for underserved areas. CircRNA-based therapeutics would benefit from increased stability and decreased expression to ensure longevity while reducing common side effects such as toxicity and immune responses resulting from overexpression of target proteins.

Awards Won:

Second Award of $2,000