Literature Review

Engineering circular RNA for potent and stable translation in eukaryotic cells

HPLC Column, Sepax, SRT SEC-2000 PEEK, 5um, 2000 A
4.6 x 300 mm
Part Number: 215980P-4630

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Massachusetts Institute of Technology

Wesselhoeft, R.A., Kowalski, P.S. & Anderson, D.G. Engineering circular RNA for potent and stable translation in eukaryotic cells. Nat Commun 9, 2629 (2018). https://doi.org/10.1038/s41467-018-05096-6

Authors

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Massachusetts Institute of Technology

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Abstract

Messenger RNA (mRNA) has broad potential for application in biological systems. However, one fundamental limitation to its use is its relatively short half-life in biological systems. Here we develop exogenous circular RNA (circRNA) to extend the duration of protein expression from full-length RNA messages. First, we engineer a self-splicing intron to efficiently circularize a wide range of RNAs up to 5 kb in length in vitro by rationally designing ubiquitous accessory sequences that aid in splicing. We maximize translation of functional protein from these circRNAs in eukaryotic cells, and we find that engineered circRNA purified by high performance liquid chromatography displays exceptional protein production qualities in terms of both quantity of protein produced and stability of production. This study pioneers the use of exogenous circRNA for robust and stable protein expression in eukaryotic cells and demonstrates that circRNA is a promising alternative to linear mRNA.

Engineering circular RNA for potent and stable translation in eukaryotic cells

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Sample

Broad Sample Type	DNA/RNA/OLIGO
Sample	circRNA
Sample Notes	1-5kb engineered circular RNA, GLuc RNA and a CVB3-GLuc-pAC splicing reaction
Molecular Weight of Sample	~340,000-2,000,000 daltons
Sample Prep	Resulting RNA fractions were precipitated with 5 M ammonium acetate, resuspended in water, and then in some cases treated with RNase R as described above.

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Experimental conditions

Column	**SRT SEC-2000 PEEK, 5um, 2000 A 4.6 x 300 mm**
Mobile Phase	RNase-free TE buffer (10 mM Tris, 1 mM EDTA, pH:6)
Gradient	isocratic
Flow Rate	0.3ml/min
Instrument	HPLC
Instrument Notes	Agilent 1100 Series HPLC, RNA was detected by UV absorbance at 260 nm, but was collected without UV detection
Injection	30ug

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Literature Reference
CircRNA on Sepax Analytical SEC

LR2022021052

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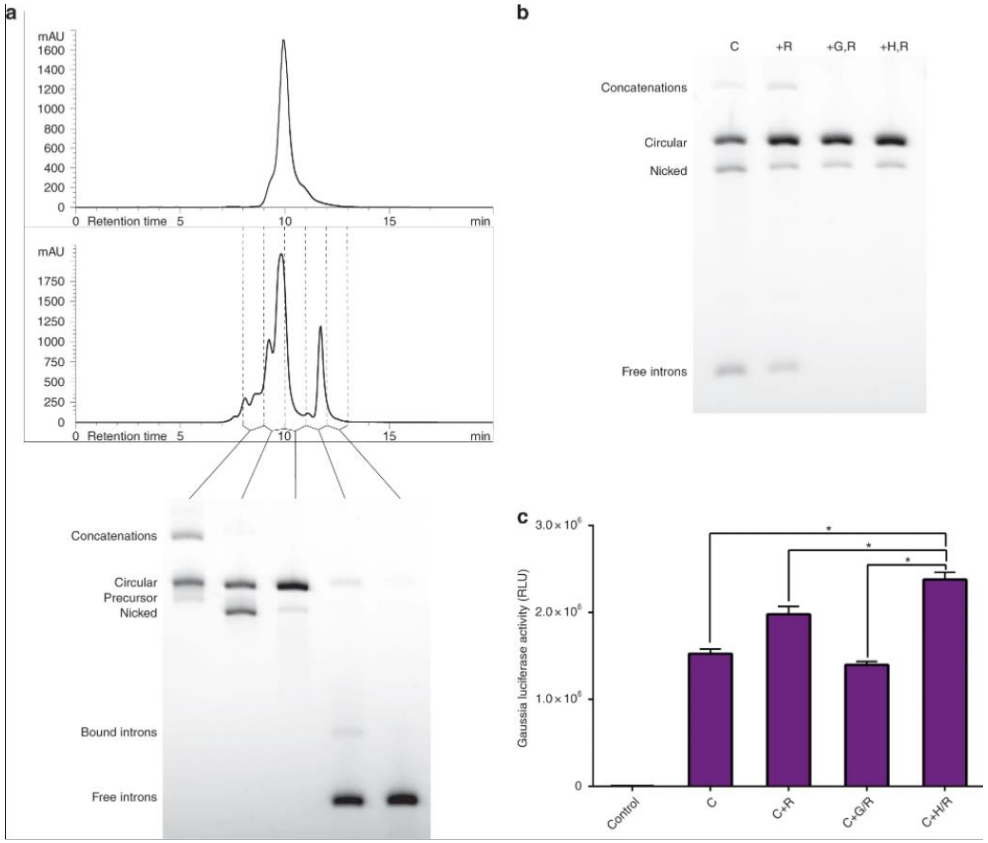

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