ROLLING CYCLE TRANSLATION OF CIRCULARIZED INFINITE OPEN READING FRAMES; FOOLING THE RIBOSOME

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Recent findings highlighting the abundance of endogenous circular RNA (circRNA) in human and mouse [1] and functional validation of numerous circRNA species [2] point towards new genetic engineering targets. Naturally occurring circRNA demonstrate greater stability over their linear mRNA counterparts, and artificial circularization of exonic sequence has been achieved [2]. More-over the translation of these molecules has now come into focus [3, 4]. In this study we sought to elucidate the potential of circular transgene open reading frames (ORF) as a means of improving translational output from an RNA molecule, using a model glycoprotein, Erythropoietin (EPO).

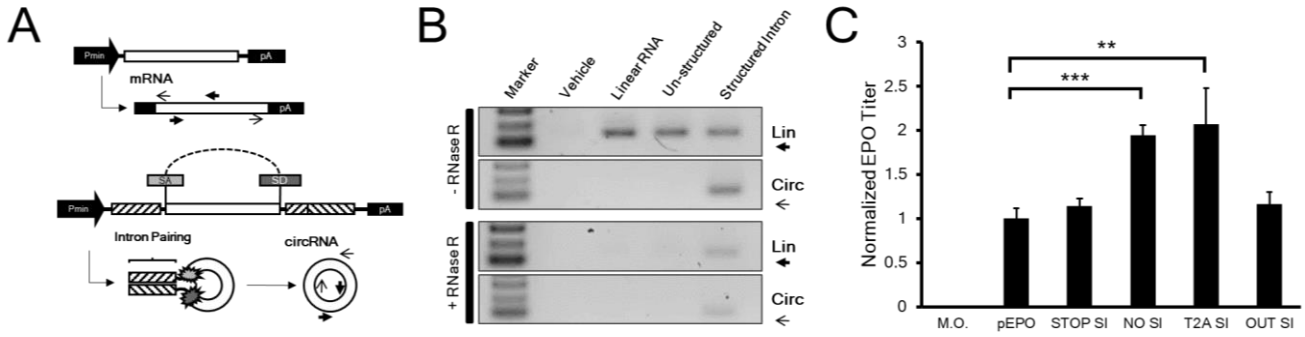

Figure 1 – RNA Circularization, Validation and Relative Product Titer; (A) Structured Intron (SI) flank aids RNA circularization. Circular and linear forms can be distinguished by convergent (Linear RNA – bold arrow) and divergent (Circular RNA – thin arrow) primer sets. Primer binding sites are represented as arrows. (B) Validation of RNA circularization by RNase R digestion. (C) Normalized EPO titer of linear control (pEPO) and circEPO constructs; STOP SI, NO SI, T2A SI and OUT SI. "M.O.", transfection reagent only control.

Artificial circularization of gene ORF is achieved by splice signals, brought in close proximity via complementary intronic flanking sequence, Figure 1 (A). circRNA is said to be RNase R resistant, [1-3]. To validate the circularization of the EPO ORF in our system, total RNA was harvested from CHO K1 transiently expressing one of; linear RNA, an un-structured intron flank or a structured intron, and treated or mock treated with RNase R prior to reverse transcription and subsequent PCR analysis. In each case, two primer sets were used, "Lin" which amplifies both the linear and circular forms, and "Circ" which amplifies the unique back-splice junction only found in the circular form, see Figure 1 (B). As anticipated we have a unique, RNase R resistant, amplicon arising from our Structured Intron facilitated circularization of EPO RNA. circRNA is a closed loop structure with no end, the notion of rolling cycle translation was investigated by the removal of the stop codon from the EPO ORF, (NO SI). To ensure correct folding and functional protein, the addition of a cleavage peptide to the endless ORF was also assessed, (T2A SI). These two constructs exhibited a 1.94 ($p=1.39x10^{-4}$) and 2.1-fold ($p=0.0034$) increase, respectively, in protein titer over a linear mRNA control, quantified by ELISA, see Figure 1 (C). The initial results are promising, and suggest circRNA could be a viable and novel addition to the CHO cell engineering toolbox.

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