環型核醣核酸增益蛋白質表現系統應用於 RNA 藥物

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摘要

本研究旨在探討環型核醣核酸（circular RNA）在人體疾病中的分子致病機制和功能角色，特別強調其轉譯活性及其在各種臨床應用中的潛力。環型核醣核酸傳統上被認為是微型核醣核酸（microRNA）的吸附分子，但特定環型核醣核酸的序列特性使其具蛋白質轉譯的活性。在我們的生物資訊工具 ACT 演算法和 circVIS 平台的協助下，我們將外顯子連接複合體（EJC）介導的環型核醣核酸轉譯機制整合到『環型核醣核酸蛋白質增益表現』(EPIC）系統中，該系統具備核醣核酸剪接誘導的非依賴帽端轉譯和可控制的亞細胞分布性。我們開發原型產品的過程使用了上下游錯接的報導基因系統，驗證正確的剪接和外顯子連接複合體的結合對轉譯是必需的。我們發現包含內含子的報導基因顯著增強了轉譯活性，與環型核醣核酸的量或是進出核變化無關。此外，將突變導入核酸剪接位點可以抑制內含子引發的增強轉譯活性，表示是核醣核酸剪接過程而非內含子序列所促進的轉譯活性。這些結果發現與展示了一種新的外顯子連接複合體介導的環型核醣核酸轉譯機制，這種機制獨立於已知的內部核糖體進入位點和核醣核酸修飾途徑。此機制對於穩定和持久的治療蛋白質表現具有重要的應用意義。通過利用環型核醣核酸的穩定性和非依賴帽端轉譯活性，我們的研究成果可以用於解決基因編輯、免疫治療和疫苗開發中面臨的持久表現挑戰。總結來說，我們的研究提供了對環型核醣核酸轉譯能力的關鍵新資訊，為治療應用提供了新的替代途徑。這些發現增強了我們對核醣核酸生物學的理解，並為治療各種人類疾病的創新策略提供了新的方向。

Enhanced Protein Expression in CircRNA as RNA Therapeutics

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Abstract

The purpose of this study is to explore the mechanisms and functional roles of circular RNAs (circRNAs) in human diseases, with a particular emphasis on their coding potential and involvement in various application contexts. CircRNAs, traditionally recognized for their role as miRNA sponges, possess unique attributes that make them feasible for protein translation. Facilitated by our computational tools, the ACT algorithm and circVIS platform, we integrated the mechanism of exon junction complex (EJC)-mediated circRNA translation into the "Enhanced Protein Expression in CircRNA" (EPIC) system, which harbors splicing-induced cap-independent translation and controlled preference for subcellular distribution. The development of our prototypic methodology involved a split reporter system to validate that proper splicing and the deposition of EJC are essential for translation. We discovered that the inclusion of an intron significantly enhances translation activity, independent of RNA levels or nuclear export changes. Furthermore, mutating the splicing site eliminated the enhanced translation, indicating that the splicing process, rather than the intronic sequences, is crucial. The results revealed a novel EJC-mediated mechanism for circRNA translation, adding to the known pathways involving internal ribosome entry site and RNA base modifications. This mechanism has significant implications for the stable and prolonged expression of therapeutic proteins. By leveraging the stability and cap-independent translation capabilities of circRNAs, our research aims to address the persistent expression challenges faced in gene editing, immunotherapy, and vaccine development. In conclusion, our study provides critical insights into the translational capabilities of circRNAs, offering new avenues for therapeutic applications. These findings enhance our understanding of RNA biology and pave the way for innovative strategies in treating various human diseases.