13. Structural and thermodynamic insights into the PAZ/3'-overhang interaction to improve siRNA specificity

Andreia F. Jorge,[#] Adele Alagia, Anna Aviñó, Santiago Grijalvo, Carme Fàbrega Alberto Pais[#] and Ramon Eritja

Dpt of Chemical and Biomolecular Nanotechnology. Institute for Advanced Chemistry of Catalonia (IQAC), CSIC, Barcelona, Spain. CIBER-BBN Networking Center on Bioengineering, Biomaterials and Nanomedicine, Barcelona, Spain.[#]CQC, Department of Chemistry, University of Coimbra, Rua Larga, 3004-535. Coimbra, Portugal.

The discovery of the RNA interference (RNAi) pathway and the identification of the small interfering RNA (siRNA) molecule as RNAi trigger,[1] have simplified the study of gene function paving the way to the treatment of any disease-related gene.[2] The siRNA molecules are short double-stranded RNAs, of approximately 21 nucleotides (nt) in length, bearing 2-nt overhangs at both 3-ends. The siRNA molecule is initially incorporated into the RISC (RNA-Induced Silencing Complex) in double stranded fashion, then a process called RISC maturation results in the dissociation of siRNA passenger strand from the guide strand, permitting the recognition of target messenger RNA (mRNA). The process of siRNA loading requires the action of the RISC-Loading Complex (RLC), which includes DICER, Argonaute2 (Ago2) and TRBP (HIV-1 TAR RNA Binding Protein) proteins. The Ago2 protein is a cradle-shaped protein of four main domains: MID (middle), PIWI (P-element-induced wimpy testes), PAZ (Piwi/Argonaute/Zwille) and N-terminal. Herein, we conduct a systematic study of Ago2 response by introducing chemical modifications at siRNA 3'-overhang by both computational and experimental techniques. Atomistic simulations and free energy calculations allow us to propose a robust and self-contained procedure for studying the factors governing PAZ/siRNA interactions. Results from calculations based on two different crystal structures allow direct comparison and consistency checks. An overall description of the systems is thus achieved, which is followed by a comprehensive experimental study on the 3'-overhang structural requirements for the design of more specific siRNA molecules.[3]

Work supported by grants CTQ2017-84415-R, 2017SGR114, CIBER-BBN (CB06/0019). A. F. J. and T. F. G. G. C. acknowledge Fundaçao para a Ciência e Tecnologia (FCT), Portugal, for financial support regarding the Post-Doctoral grant SFRH/BPD/104544/2014. CQC is supported by FCT through projects PEst-OE/QUI/UI0313/2014 and POCI-01-0145-FEDER-007630.

1. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001, 411, 494-498.
2. Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. Chem Biol. 2012, 19, 60-71.
3. Alagia, A. Jorge AF, Aviñó A, Cova TFGG, Crehuet R, Grijalvo S, Pais AACC, Eritja R. Exploring PAZ/3'-overhang interaction to improve siRNA specificity. A combined experimental and modeling study. Chem. Sci., 2018, 9, 2074-2086.

14. CRISPRing zebrafish to understand early vertebrate development and human diseases

Miguel A. Moreno-Mateos[1,2]

[1] Department of Genetics, Yale University School of Medicine, New Haven, CT, USA. [2] Fundacion Progreso y Salud, Seville, Spain. e-mail: moreno.mateos.ma@gmail.com

CRISPR-Cas9 system is a powerful genome engineering approach that is now widely used. This targeting system is based on two components: a single guide RNA (sgRNA) that directs the Cas9 endonuclease to the target site to be mutated However, variable activity across different sgRNAs can limit the mutagenic efficiency. We have optimized the CRISPR-Cas9 system in zebrafish by analyzing the mutagenic activity of 1920 sgRNAs and developing an algorithm, CRISPRscan (www.crisprscan.org) that efficiently predicts sgRNA activity in vivo. This and other optimizations have allowed us to perform functional genetic screens in vertebrates in a rapid and efficient manner identifying a novel protein complex involved in splicing and vertebrate brain development that is found mutated in patients with neurodevelopmental disorders. Together, these results provide novel insights into the determinants that mediate CRISPR-Cas9 efficiency and its application to uncover genes involved in human diseases and developmental disorders.