Cytosolic CRISPR RNAs for efficient application of RNA-targeting CRISPR-Cas systems

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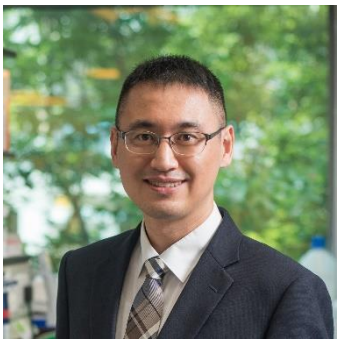

Abstract

Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) technologies have evolved rapidly over the past decade with the continuous discovery of new Cas systems. In particular, RNA-targeting CRISPR-Cas13 proteins are promising single-effector systems to regulate target mRNAs without altering genomic DNA, yet the current Cas13 systems are restrained by suboptimal efficiencies. Here, we show that U1 promoter-driven CRISPR RNAs (crRNAs) increase the efficiency of various applications, including RNA knockdown and editing, without modifying the Cas13 protein effector. We confirm that U1-driven crRNAs are exported into the cytoplasm, while conventional U6 promoter-driven crRNAs are mostly confined to the nucleus. Furthermore, we reveal that the end positions of crRNAs expressed by the U1 promoter are consistent regardless of guide sequences and lengths. We also demonstrate that U1-driven crRNAs, but not U6-driven crRNAs, can efficiently repress the translation of target genes in combination with catalytically inactive Cas13 proteins. Finally, we show that U1-driven crRNAs can counteract the inhibitory effect of miRNAs. Our simple and effective engineering enables unprecedented cytosolic RNA-targeting applications.

Biography

Chul Kwon is an Assistant Professor at the University of Hong Kong (HKU). Before joining HKU in 2020, he studied microRNAs and RNA-binding proteins in Narry Kim's lab at Seoul National University in Korea. He provided a whole RNA-binding protein repertoire of embryonic stem cells in collaboration with Matthias Hentze's lab at EMBL in 2013. After that, he revealed the molecular mechanism of DROSHA, a key enzyme in microRNA biogenesis, using structural biology and high-throughput biochemistry. He also identified ERH as the third component of Microprocessor, which was believed to be comprised of DROSHA and DGCR8 for over the decade. With his own lab at HKU, he is developing new RNA-related technologies to deepen our understanding of RNA regulations and correct abnormal gene expression in diseases.