Poster

Expression and Purification of Cas13d

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ABSTRACT

The class 2 type VI CRISPR Cas13 systems, an RNA targeting CRISPR-Cas effector, has been originally involved in adaptive prokaryotic immunity by protecting bacteria against invading RNA phages, It is currently used as a tool to cut and degrade RNA in a precise manner in yeasts, mammalian and plant cell lines. In the Moreno-Mateos lab the CRISPR-RfxCas13d system has been recently shown as an efficient and specific system in zebrafish and other models targeting mRNA in animal embryos (Kushawah et al., Biorxiv 2020). Our lab is interested in understanding an early embryogenesis process called the maternal-to-zygotic transition (MZT). This process implies the activation of a silent embryonic genome by the maternal mRNA products deposited in the oocyte. We have used our CRISPR-Cas13d technology to knockdown mRNAs from the maternal contribution with a role in the zygotic genome activation (ZGA). For example, we injected mRNA coding Cas13d and gRNAs targeting nanog, a maternally-provided factor crucial for the ZGA, and we observed classical phenotypes when MZT is altered. However, we found that the penetrance of the targeting and the observed knockdown phenotypes can be optimized. To achieve this, we reasoned that the injection of the purified protein instead the mRNA coding Cas13d could have an earlier targeting of these and other maternal RNAs.

In this work, we have generated a bacterial expression vector and successfully purified RfxCas13d endonuclease. The RfxCas13d protein was expressed in E. coli cells and purified by affinity and ion-exchange chromatography methods. In vivo tests in zebrafish embryos demonstrate that the purified protein is biologically active and the complex Cas13-gRNA triggers efficient mRNA knock-down showing higher targeting RNA depletion than injections of Cas13 mRNA. We are currently analysing the specificity of the Cas13d protein in vivo by RNAseq experiments and performing in vitro cleavage assays to complement the results obtained in vivo.

All together, these results demonstrate that the use of purified protein for maternal RNA targeting is an efficient and convenient system to potentially uncover novel maternal RNA functions. This new optimized tool will open up new avenues that will illuminate our knowledge in early vertebrate embryogenesis.

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