Optimization of CRISPR-Cas13d in vivo

Dahiana E. Monges, Luis Hernández-Huertas, Miguel A. Moreno-Mateos

CABD, Centro Andaluz de Biología del Desarrollo, Sevilla, España *

Abstract

The class 2 type VI CRISPR Cas13 system, an RNA targeting CRISPR-Cas effector, has been 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 also 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 maternal RNAs with a role in the zygotic genome activation (ZGA). With the current methods, injecting mRNA coding Cas13d and gRNAs targeting *nanog*, a maternally-provided factor crucial for the ZGA, 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 of the mRNA coding Cas13d could have an earlier targeting of these and other maternal RNAs and therefore increase the phenotype penetrance. 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. RfxCas13d did not show any toxicity in zebrafish embryos and we observed a faster targeting correlating with more extreme maternal phenotypes. All together, our results demonstrate that using RfxCas13d purified protein is an optimized method to interrogate maternal RNA function in a systematic manner.

1 Aim: understand MZT

A

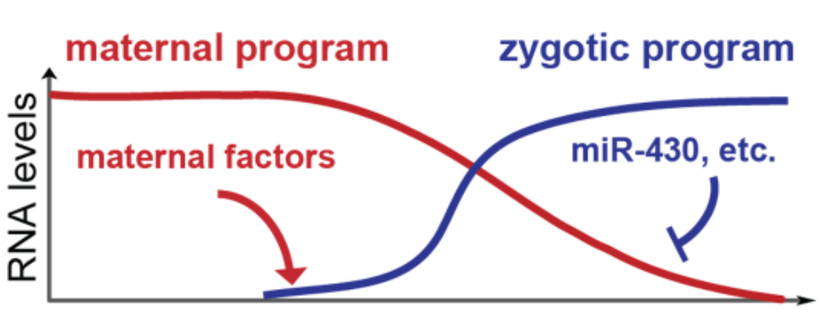


Figure 1: A. Maternal to zygotic transition. We try to understand the molecular mechanisms by which some factors lead to activate transcription for first time after fertilization, a process called zygotic genome activation (ZGA). The goal is to optimize a RNA targeting method in vivo to uncover new maternal factors involved in the ZGA.

2 Protein purification

A

B

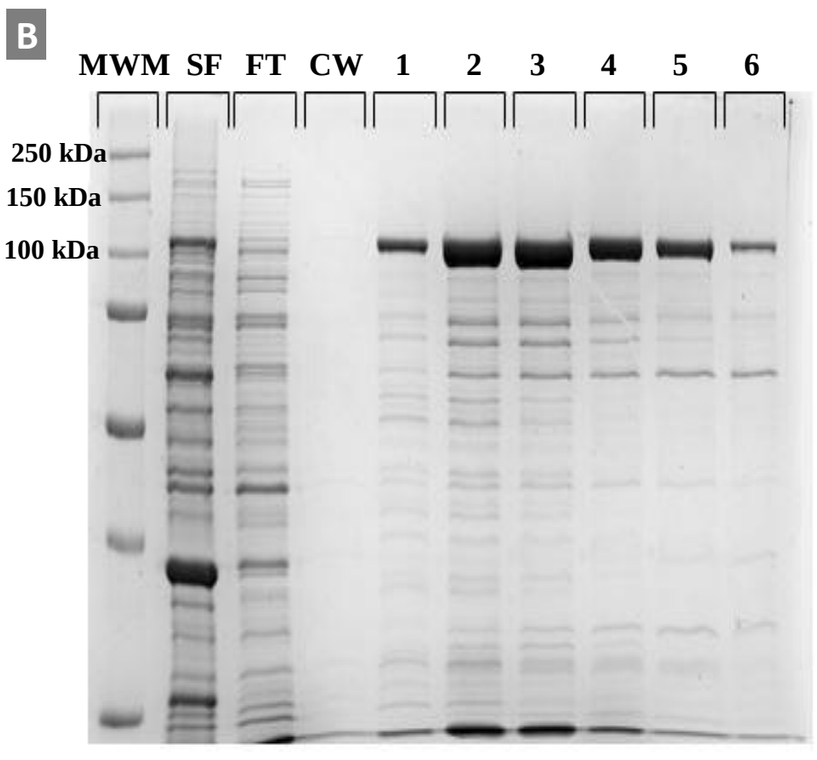
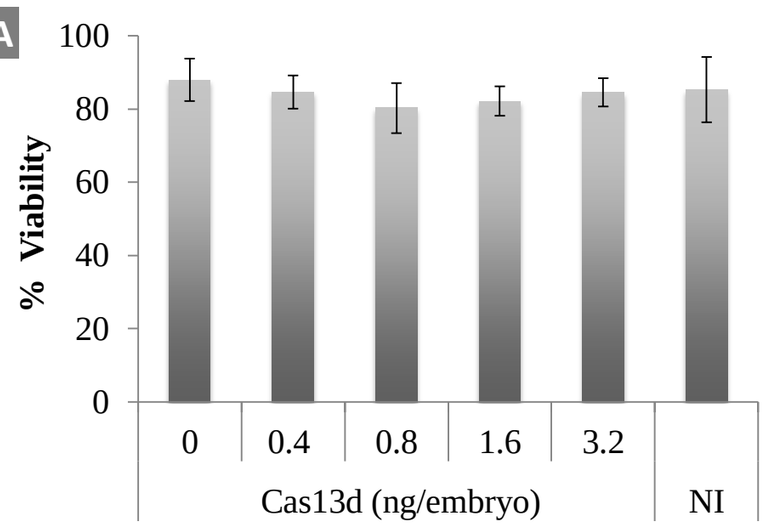


Figure 2. Protein purification. A. Expression vector encoding RfxCas13d**. B.** SDS–PAGE gel of RfxCas13d protein purification. Coomassie blue-stained 10% SDS–PAGE. MWM, protein molecular weight marker (Precision Plus Protein All Blue Standards, BioRad); SF, soluble fraction of induced-cell lysate; FT, HisTrapFF column flow through; CW, column washes; lines 1, 2, 3, 4, 5, and 6, elution of the His-tagged protein with 50 to 150 mM imidazole.

3 Toxicity evaluation of embryos

A



B

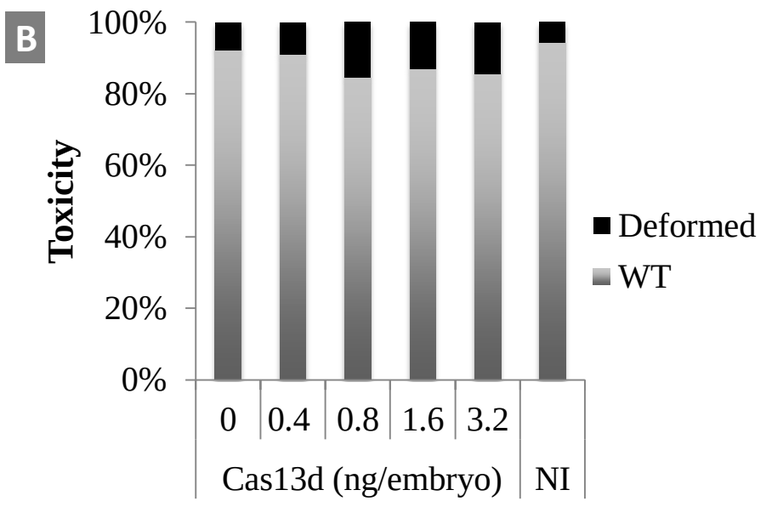
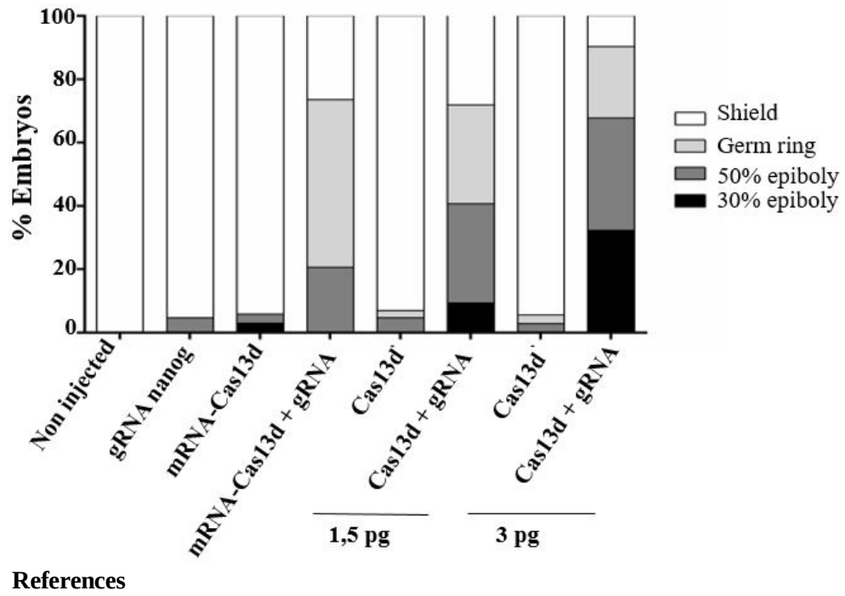


Figure 3: Toxicity evaluation of embryos. A. Different concentration of RfxCas13d was injected into one-cell stage zebrafish embryos to analyze toxic effects. **B**. The graphic shows the percentage of deformed and wild type (WT) zebrafish embryos 24 hours post fertilization, hpf.

4 Protein RfxCas13d vs mRNA RfxCas13d activity

A



References

Kushawah et al.. CRISPR-Cas13d induces efficient mRNA knock-down in animal embryos. BIORXIV https://www.biorxiv.org/content/10.1101/2020.01.13.904763v1

(CONTINUED)

B

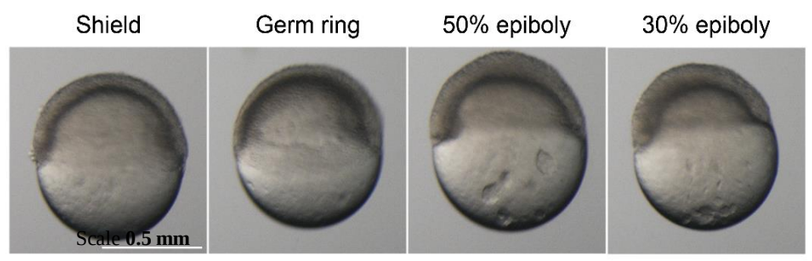


Figure 4: RfxCas13d vs Protein RfxCas13d. A. Phenotypic severity in embryos injected with RfxCas13d mRNA, RfxCas13 protein and gRNAs targeting *nanog* under various injection conditions compared to non-injected embryos evaluated at 6 hours post fertilization (hpf) showing percentage of observed phenotypes. **B**. Pictures of developmental stages from embryos injected with gRNAs targeting *nanog* at one-cell stage.

5 qPCR *nanog*

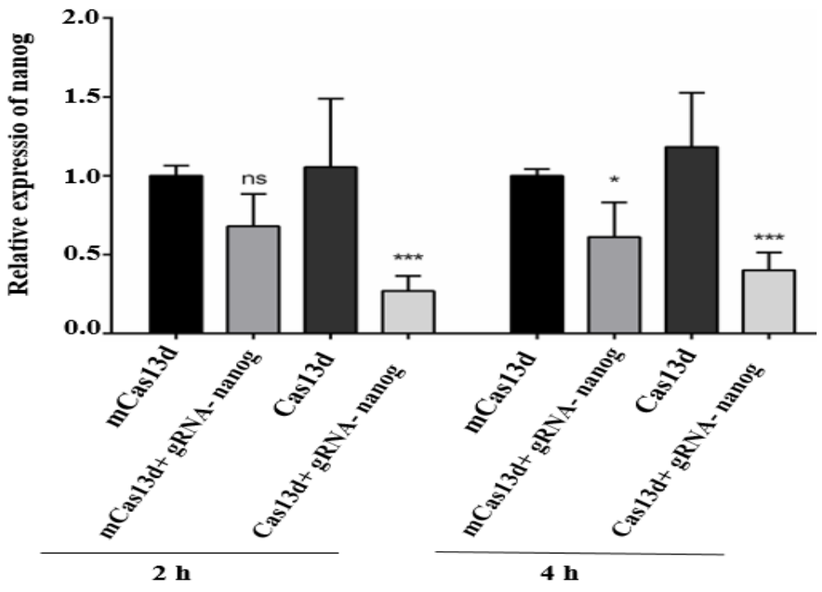


Figure 5. qPCR *nanog*. RT–PCR analysis showing levels of *nanog* mRNA in embryos at 2 and 4 hpi injected with RfxCas13d protein or mRNA alone or together with gRNAs targeting *nanog* mRNA. T-test were use to compare the results from mRfx-Cas13d and Rfx-Cas13d protein knockdown activity .Where NS means = no significants; * (P value=0.05 and *** (P value= 0.001) respectively.

Conclusions

- RfxCas13d protein can be efficiently purified in *E. coli*.
- The use of purified Cas13d protein increases CRISPR-Cas13d activity in vivo during early development without showing any toxicity.
- This optimized system is specially indicated for maternally provided RNA targeting.

Acknowledgement

Members of Moreno-Mateos Lab; Aquatic Vertebrate Facilities; Proteomics Service; CABD Springboard Program; Ramón y Cajal Program 2017-ryc-2017-20041, aid pp 2019 for the development of UPO`s own research lines; Project of the Science Ministry PGC2018-097260-B-100.