Poster

Optimization of CRISPR-Cas systems in ectothermal organisms

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ABSTRACT

CRISPR-Cas systems have been traditionally optimized for gene editing in different types of models, such as in mammalian cell lines. However, their implementation in animal models requires additional work. In addition, another impediment is the number of genomic targets of CRISPR-Cas systems since it is limited due to PAM (Protospacer adjacent motif) sequence restriction. PAM sequence is a short DNA sequence, characteristic of each CRISPR-Cas system, near to the target DNA region and required for each Cas nuclease to recognize the target and cut it. Therefore, the possibility of having different CRISPR-Cas systems with different PAM sequences allows us to have a greater number of targets in the genome, which would simplify and increase our possibilities of carrying out a gene editing anywhere in the genome and in a more precise manner.

In the laboratory we are interested in implementing new CRISPR-Cas systems in vivo such as CRISPR-Cas12b and a Type I CRISPR-Cas, using zebrafish embryos as a model in animals. First, we are optimizing CRISPR-Cas12b system that has been recently characterized in cell culture (1,2) and allows gene editing in genome locations rich in AT regions where other endonucleases already characterized such as Cas12a or Cas9 cannot target. Optimizing this system in zebrafish embryos will increase the number of genomic targets that can be used in vivo. Second, type I CRISPR-Cas system is formed by Cas3 and a ribonucleoprotein complex (RNP) called Cascade. This system has been able to trigger long-range deletions (3) but its activity in vivo has not been shown yet. Therefore in this project we are implementing type I CRISPR-Cas tool in vivo to facilitate the elimination of large regions in the genome with potential role during embryogenesis.

To evaluate the activity of both CRISPR-Cas systems, we are generating different expression vectors by cloning, to subsequently obtain the mRNA of the protein. On the other hand, we are designing specific guide RNAs (gRNAs) for these systems whose targets are genes involved in the pigmentation of zebrafish that facilitates the analysis of these CRISPR-Cas systems. We are currently co-injecting mRNAs and gRNAs of each system in the zebrafish embryos and evaluating the in vivo activity of each system. All together, we will optimize novel CRISPR-Cas systems that will be used for precise gene editing in vivo.

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