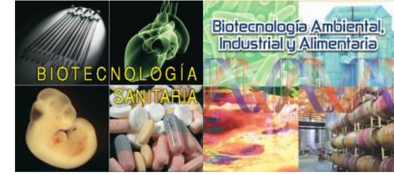
Talk



CRISPRing zebrafish to understand early vertebrate development and human diseases

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ABSTRACT

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 developing an algorithm, CRISPRscan (www.crisprscan.org) that efficiently predicts sgRNA activity in vivo. Using these optimizations we have uncover 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.

However, the number of genomic targets of the CRISPR-Cas9 system is limited due to the PAM sequence restriction. To extend the in vivo repertoire of potential targeting sites in the genome, we have characterized and optimized different CRISPR associated endonucleases such as AsCpf1 and LbCpf1. We have demonstrated that temperature modulates Cpf1 activity being this effect stronger on AsCpf1 explaining its lower activity in ectothermic organisms such as Drosophila, Xenopus and zebrafish. These results contribute to the molecular understanding of Cpf1 activity in vivo and establish this tool as an efficient genome engineering system across ectothermic species.

Finally, one of the main interests in the early development field is to study the maternal-to-zygotic transition, a universal process that occurs in all animals. One of the critical stages during this transition is the activation of the silent zygotic genome after fertilization but the mechanisms underlying this process are poorly understood. To address this, we have optimized a CRISPR-Cas9-based live imaging approach of transcription in zebrafish. We show that genome activation does not require the titration of maternal repressors, occurs independent of cell division and is regulated through the effects of maternally-provided factors on the chromatin acetylation. In summary, these results open a new dimension into understanding the maternal factors and mechanisms that enable activation of the genome.

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