The use of CRISPR- molecular scissors to study gene function

Marta Oliveira

Targeting and cutting DNA is possible and allows the modification of model organism genome. In this case, the CRISPR-Cas technique was used to silence two key genes in kidney and vasculature development in zebrafish.

The term CRISPR (clustered regularly interspaced palindromic repeats) is used to describe a family of repetitive sequences found in Bacteria. These short repeats are regularly interspaced by unique DNA regions that come from several bacterial viruses, which works as a DNA record of previous attacks. It was later proved that the CRISPR loci, together with many enzymes, were in fact a bacterial defense mechanism against life threatening viral attacks, preventing the virus from thriving by cutting their DNA.
The Cas9 (CRISPR associated) enzyme is the DNA cutting enzyme – the scissors- of one particular bacteria species (*Streptococcus pyogenes)* which recognizes the DNA target with the help of a CRISPR RNA. This RNA is generated from the CRISPR loci matching to the target viral DNA and binds to it by base-pair complementarity, leading the Cas9 scissors to the DNA target.
This bacterial immune system was modified to target and cut specific, desired locations of DNA in other organisms such as human cells, plants, mice and zebrafish. This was achieved by matching a Cas9 enzyme with a synthetic, specially designed, guide RNA and introducing them into the organism where the gene editing is desired to occur. After the Cas9 generates a cut on the gene of interest natural repair mechanisms are triggered to fix it, and by doing so, they unpredictably add or remove pieces of that gene, mutating it. Therefore, this new tool can be used to disturb and silence any gene of interest, allowing researchers to know more about the function of the proteins they code for by studying what happens to an organism when certain proteins are not present.
Pdgfrb and Foxc1a are two proteins with a function in vasculature and kidney development. Inactivating these genes will bring insights on their role in these systems, as well as help understand their role in several important molecular pathways. By injecting the two components (Cas9's and guide RNA) in newly fertilized zebrafish embryos with a fine needle (and letting them develop), we were aiming to target the mentioned genes and generate mutants, this is, individuals with a mutation.
It was possible to induce mutations in the *pdgfrb* and *foxc1a* genes in one of the two copies of the gene. They do not show any abnormal phenotype, because the healthy, unmodified allele is still functional, and therefore is compensating for the mutated, silenced one. However, if they transmit the mutated allele to their offspring, 25% of them will have both mutations in both copies of their gene. These mutants will be used to study protein function.
The CRISPR Cas9 system is still poorly known, and there are some problems of this technique, such as the possibility of cuts at unintended locations in the genome, already observed by some researchers. However, the increase of knowledge about the CRISPR-Cas9 system, will lead to more flexibility and ease of use in this recently popular genome engineering tool. At this point, CRISPR-Cas9 shows the potential to be a truly transformative technology, by significantly accelerating the rate of discoveries in all areas of biology including medicine, agricultural goods and gene function.

Degree project in Biology, Master of Science (2 years), 2014
Examensarbete i biologi 45 hp till magisterexamen, Uppsala universitet, 2015
Biology Education Center and Dept. Immunology, Genetics and Pathology, Rudbeck Laboratory
Supervisor: Dr. Lwaki Ebarasi