Delivery of CRISPR/Cas9 into the mosquito germline by receptor mediated endocytosis
Duverney Chaverra-Rodriguez

II. Abstract

Cas9-mediated gene editing is a powerful technique for addressing research questions in arthropods of medical importance. Current approaches rely upon delivering enzymes, DNA, and RNA to preblastoderm embryos via embryonic microinjection. However, embryonic microinjection is technically challenging, is limited to a small number of arthropod taxa, and is inefficient even in optimized species. As such, there is a critical need to develop methods for Cas9 delivery that are simple, accessible for many researchers and generally compatible for a large variety of arthropod species. Here, I propose to deliver Cas9 into the mosquito oocytes via maternal injections. I identified a peptide ligand (P2C) that binds specifically to mosquito oocyte receptors, and I demonstrated that when fused to enhanced green fluorescent protein (EGFP), it is internalized when injected in the hemolymph of females of seven mosquito species during oocyte development. These results suggest P2C can be used to deliver other cargo into the oocytes. In this proposal, I will evaluate the ability for P2C to transduce Cas9 to mosquito ovaries. The optimization of Cas9 delivery via maternal injections will benefit researchers who wish to easily perform genome editing in the germline of mosquitoes and other disease vectors.