IGTRCN Peer-to Peer Training Fellowship Report

Project title: Active genetics to fight against Chagas Disease

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Host Lab: Dr. Ethan Bier, UCSD, California

Period of visit: March 2017 – July 2017

Objectives of training

The goal of this training was to develop an efficient gene drive using CRISPR-Cas9 in the hemipteran model *Rhodnius prolixus*. This kissing bug is one of the main vector of Chagas Disease in Central and South America, a disease that affects around 8 million of people [1]. This disease brings terrible lifelong consequences to affected individuals and ultimately death. There are only 2 drugs against the parasite *Trypanosoma c*ruzi, and usually patients have diverse side effects with prolonged use necessary for the treatment.

The main control method against the insect is spraying insecticide inside houses to eliminate triatomine bugs. However kissing bugs insecticide resistant populations have been found in several countries [2] , which requires new technologies to fight against Chagas disease transmission.

With the new gene drive technology developed in Dr. Ethan Bier lab (Mutagenic Chain Reaction or MCR) our aim is to generate effective CRISPR transgenesis in this kissing bug, as a first step to develop gene drive constructs that will help to fight against Chagas Disease. This recently developed technology has three basic elements: (1) the cas9 gene being expressed in the germline and somatic cells; (2) the guide RNA (gRNA) that direct the cas9 to a target genomic region of interest and (3) the homology arms flanking both sides of the genomic break generated by cas9 enzyme [3].

The MCR consists of the integration of Cas9 and the gRNA on the genome, and thus, new sequences introduced in any organism can bypass the Mendelian laws of Inheritance. Using this powerful tool, we believe that we can insert and drive the expression of anti-parasite gene in *R. prolixus*, making the kissing bugs refractory to the infection by the parasite *T. cruzi*, and thus preventing the kissing bugs to infect new hosts.

Outcome of training:

Objective 1: Identify and isolate promoter sequences to direct Cas9 expression in the germline:

Our group already had transcriptomic and *in situ* hybridization data about genes that are expressed specifically in the germline of *R. prolixus*. Using this information, and taking advantage of the facilities provided by the laboratory of Dr. Ethan Bier, I re-sequenced the genomic loci as well as used RACE PCR to define exactly the start and stop codon of these

genes. The promoter sequence chosen to direct Cas9 expression in the germline was of *Rp-vasa*. The upstream sequence was cloned using TOPO cloning vector, and later joined with the Cas9 coding sequence using the Gibson Assembly method.

Objective 2: Define appropriate genomic regions for the genomic integration of the transcript.

Searching for possible landing site of the MCR construct, we tested by interference RNA (RNAi) the function of several genes that already are used as phenotypic markers in other insects. Analyzing the phenotype of injected female's progeny, we selected as landing site one of these genes that displayed an eye pigment phenotype. Therefore, I designed gRNA targeting this genomic locus to induce double-stranded break disrupting coding sequence. At the same time, I designed primers to amplify the genome sequence, adjacent to the cut site, that will be used as homology template to induce Homology-Directed Repair (HDR) in the final cassette.

Objective 3: Define promoter upregulated upon blood feeding.

To engineer resistance to *T. cruzi* in transgenic *R. prolixus*, we searched for genes that are upregulated upon blood feeding. These sequences would be used to direct the expression of effector molecules, targeting the parasite in the gut of the insect. Analysis of published transcriptome data directed our search [4]. The promoter sequence was cloned and fused with anti-parasite cassette, hoping to drive the expression of effector molecules under control of blood responsive elements, when present as a transgene.

Objective 4: Design constructs for the expression of effector proteins in the *Rhodnius prolixus.*

In order to construct the final MCR construct, all sequences described above were assembled into the final cassette containing the effector molecules. The effector molecules were synthesized by Genscript, Inc. and they were put under control of one blood responsive sequence (Objective 3). With the introduction of this effector molecules in the *R. prolixus* genome, we expect the clearance of the viable *T. cruzi* in *R. prolixus* gut, and consequently the reduction of Chagas disease transmission in endemic areas.

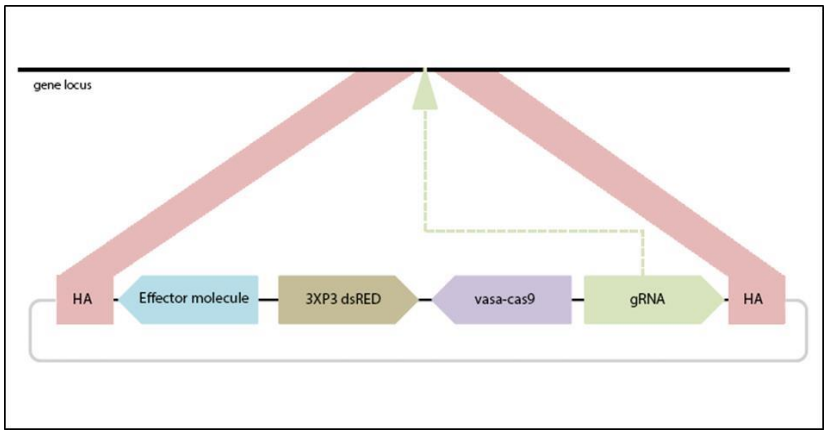

Figure 1. *R. prolixus* MCR construct carrying effector molecule against *T. cruzi*, developed during my training. HA = homology arm.

Next steps

All constructs designed during my training will be taken to Brazil, where we will proceed with the following stages that include: the transformation and analysis of effectiveness of MCR in *R. prolixus* model. Once we test these constructs for integration and efficiency they will be made available to the scientific community.

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1. WHO, *Chagas Disease (American trypanosomiasis)* in *Fact sheet* 2013.
2. Vassena, C.V., M.I. Picollo, and E.N. Zerba, *Insecticide resistance in Brazilian Triatoma infestans and Venezuelan Rhodnius prolixus.* Med Vet Entomol, 2000. **14**(1): p. 51-5.
3. Gantz, V.M. and E. Bier, *Genome editing. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations.* Science, 2015. **348**(6233): p. 442-4.
4. Ribeiro, J.M., et al., *An insight into the transcriptome of the digestive tract of the bloodsucking bug, Rhodnius prolixus.* PLoS Negl Trop Dis, 2014. **8**(1): p. e2594.