Using CRISPR/Cas9 Genome Editing to Knockout MHC Class I in *Xenopus laevis*

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ABSTRACT

The immune system of the frog *Xenopus laevis* is similar to humans. MHC Class I is a vital molecule for the immune system of *Xenopus laevis*. It presents peptides to CD8 T-cells and the presentation of self peptide fragments is crucial for immune self recognition. When MHC Class I presents non-self peptide fragments, it triggers an immune response, causing CD8 T-cells to kill the infected cells. All cells express some level of MHC Class I because all cells can be infected. The role this molecule plays in immune function and self recognition is of particular interest in *Xenopus laevis* since tadpoles are immunocompetent, yet have undetectable levels of MHC Class I protein (mRNA can be detected in different tissues). MHC Class I protein levels become detectable after metamorphosis and are expressed both as mRNA and protein in adult frogs. We are interested to see if MHC Class I is critical for immune function in *Xenopus laevis* tadpoles and will investigate by knocking out the MHC Class I gene. To do this, we utilized the CRISPR/Cas9 gene editing tool. Cas9 creates a break in the dsDNA at the location of the gene by using specific guide RNAs, and while the cell attempts to fix its genome multiple insertions and/or deletions can occur in the sequence that inactivates the gene. We generated transgenic tadpoles that have guide RNAs targeting the MHC class I gene. Currently, we are using DNA sequencing to verify successful knockout of the MHC Class I gene.

INTRODUCTION

Xenopus laevis is used for these experiments as it is a good model organism. Female frogs can be injected with hCG hormone and stimulated to produce eggs. These eggs are also easily manipulated as they are laid externally. This allows them to be injected with transgenic constructs and reagents right after fertilization. Importantly, Xenopus has an immune system that resembles the mammalian immune system which allows for comparisons between the two systems. We study the MHC class I protein, a crucial part of the immune system. It functions in self recognition and allows immune cells to recognize infected or abnormal cells. This is of particular interest in the *Xenopus laevis* model as MHC class I protein is undetectable in tadpoles, however, it is present in adult frogs. This is interesting, especially since the tadpoles are immunocompetent. Since both the adult frogs and tadpoles are immunocompetent, it is unclear as to the role that MHC class I protein has in the frog's immune system. In order to study MHC class I, CRISPR Cas9 gene editing was used to target the genes which code for the α_1 and α_2 domains of the molecule. These genes were targeted through the use of a guide RNA (sgRNA), which allowed CRISPR Cas9 to bind and generate a double-stranded break in the DNA. This double-stranded break is repaired by the cells using non-homologous end joining which introduces mutations to the gene. These mutations will make the protein ultimately nonfunctional providing insight into the function of MHC class I.

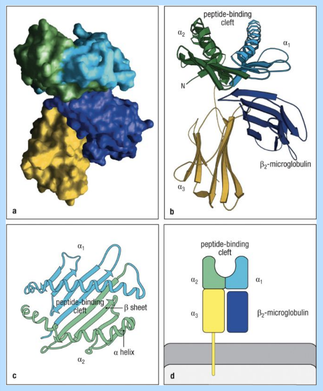

Figure 1: (A) The protein structure of MHC class I with a space filling model, (B) ribbon structure, (C) ribbon structure of the peptide binding groove and, (D) a representation of a membrane bound protein. From Janeway Immunobiology 10th edition.

RESULTS

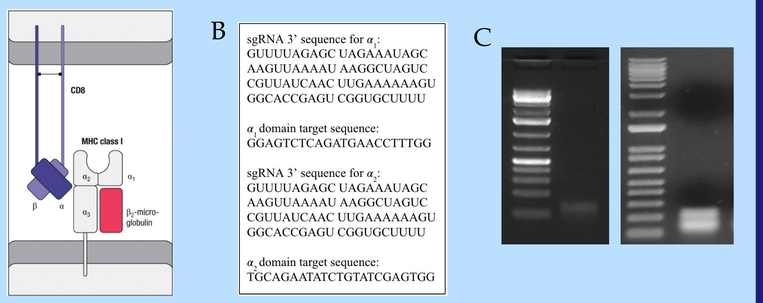

Figure 2. MHC class I protein and MHC class I sgRNA. (A) MHC class I molecule and its interaction with the CD8 co-receptor. Variable and conserved regions are shown on MHC class I (B) The sequence for the 3′ designed MHC class I sgRNA for both the α_1 and α_2 is shown as well as the target sequence in the DNA for each region. Design tool used: CRISPRdirect. (C) Left: The α_1 domain sgRNA run on an agarose gel. Right: The α_2 domain sgRNA run on an agarose gel. The lanes, left to right, contain the DNA marker and the α_1 or α_2 domain sgRNAs.

RESULTS

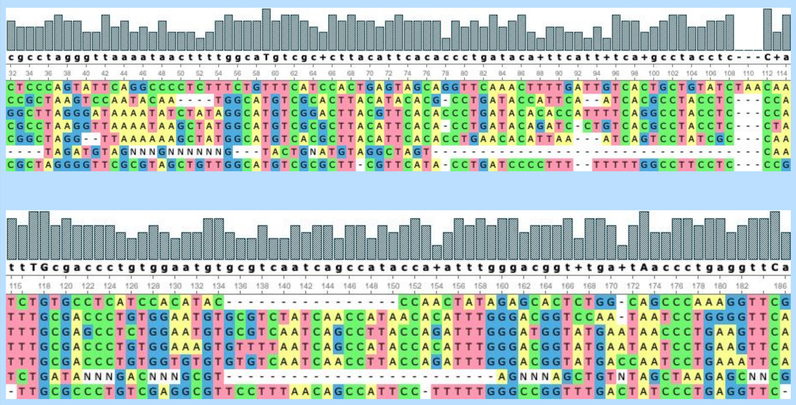

Figure 3. Genetic Sequence Alignments. Alignments of sequences of PCR samples at 30-186 bp compared to the known *Xenopus* MHC class I sequence, from top to bottom; MHC class Ia published sequence, Transgenic (TN) α_1 sample 21, TN α_1 sample 20, TN α_1 sample 1, dejellied control (DJC) sample 9, and DJC sample 8.

METHODS & MATERIALS

sgRNA Creation: sgRNAs were designed using the CRISPR direct tool (https://crispr.dbcls.jp/) and the chosen sequences were run through BLAST to confirm they matched the *Xenopus laevis* α_1 and α_2 regions. The sgRNAs were prepared with annealing PCR. The concentration and purity of the primers was confirmed using the NanoDrop Spectrophotometer and gel electrophoresis.

Transgenesis: Transgenesis was performed at the University of Rochester. Fertilized eggs were dejellied in HCL-cysteine in 0.1X MBS. The dejellied eggs were injected with 10 nL mixture of 8 ng of Cas9 protein and 200 pg/nL of sgRNA. Injected eggs and dejellied controls were compared but no difference was found in survival rates. Transgenic and control tadpoles were allowed to hatch. All tadpoles that died were used for DNA extraction.

PCR: PCR was utilized to amplify the α_1 and α_2 regions of the MHC class I gene. The resulting PCR products were run on an agarose gel to ensure the correct size and lack of contamination prior to sequencing.

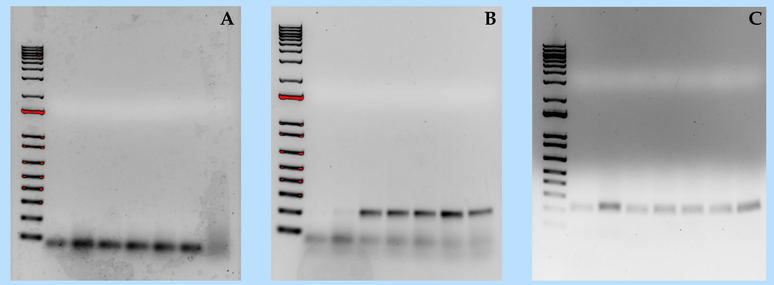

Figure 4: Gel electrophoresis experiments. The lanes from left to right show DNA ladder, water control, and six transgenic samples. (A) Gel using the α_1 primer. (B) Gel using the α_2 primer. (C) Gel using the EF-1 α primer.

CONTINUING RESEARCH

- Use gel extraction to purify PCR products
- Send DNA samples for sequencing to confirm the knockout of the α_1 and α_2 regions of the MHC class I molecules
- Analyze sequences for knockout of MHC class I
- Create more transgenic tadpoles at University of Rochester to ensure that our results are repeatable

ACKNOWLEDGEMENTS

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