

The role of mosquito miRNAs in *Wolbachia*-mediated inhibition of Zika virus



EB Dickinson, Elodie Ghedin, Denis Voronin

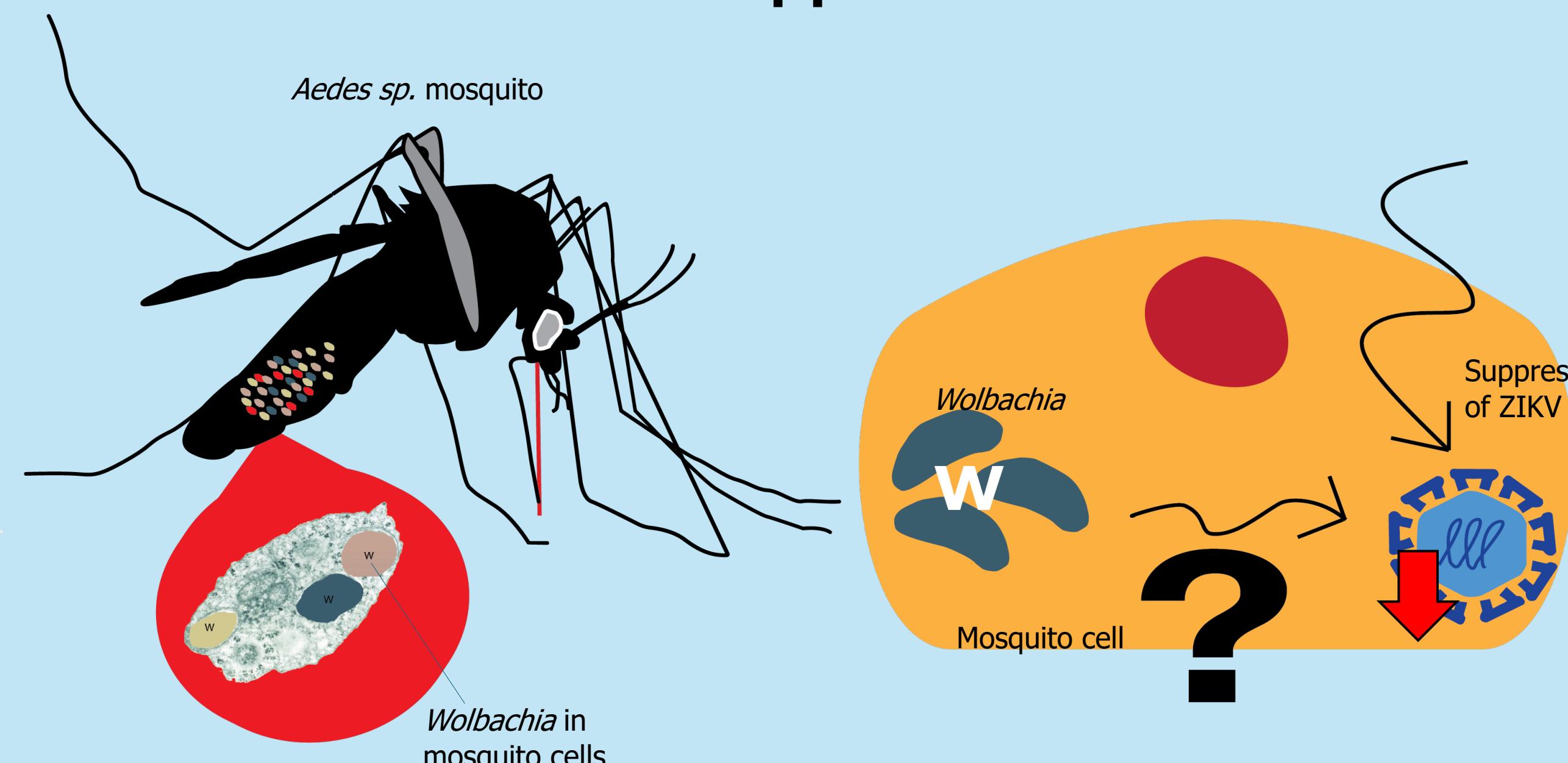
Systems Genomics Section, Laboratory of Parasitic Diseases, NIAID, NIH



Introduction

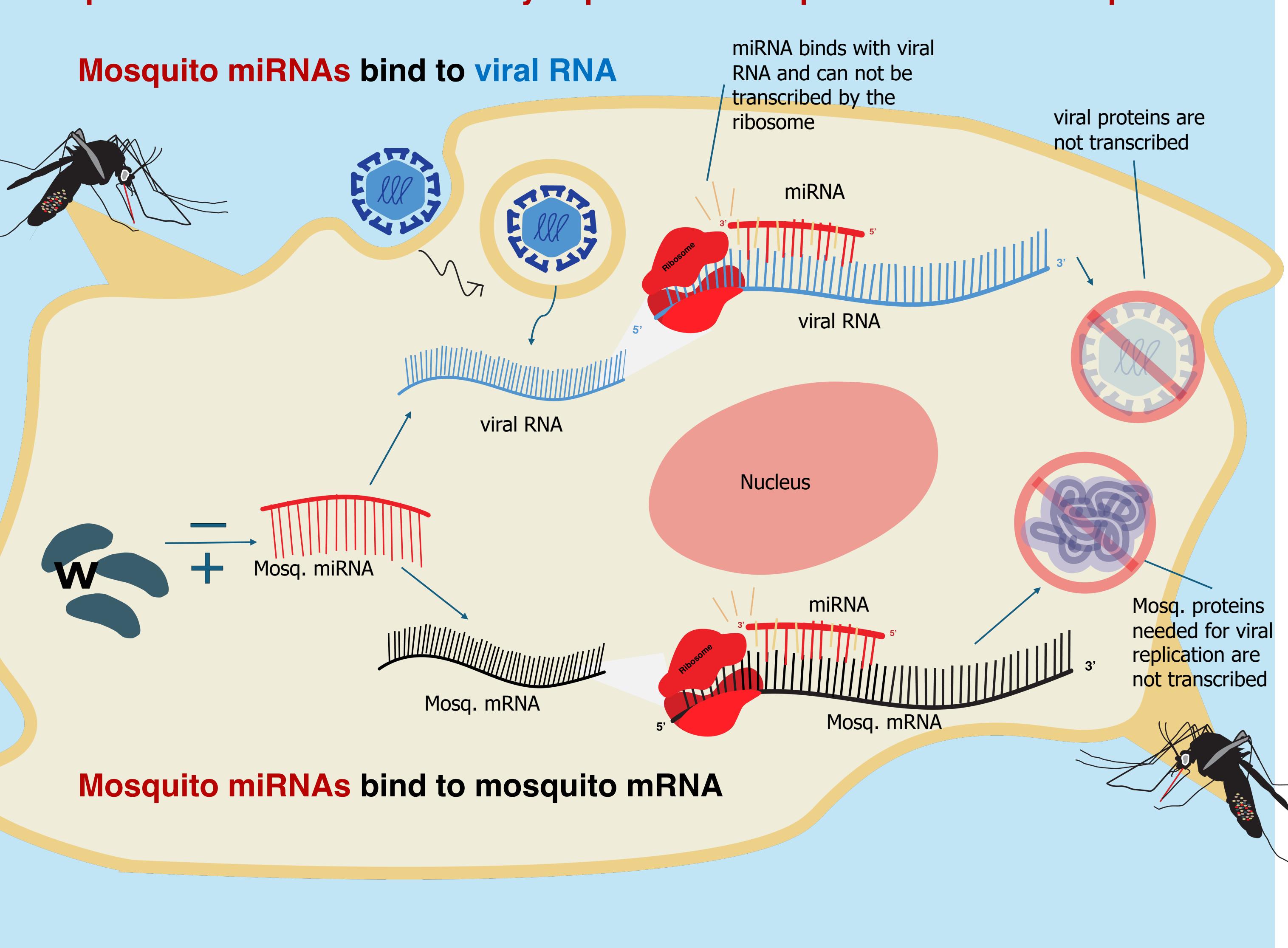
Mosquitoes are medically important vectors for pathogen transmission, including protozoa, nematodes, and viruses. Among these pathogens is the Zika virus (ZIKV), a newly emerged mosquito-borne disease associated with neurological complications. ZIKV can be transmitted from mother to fetus, causing severe birth defects such as microcephaly, which leads to abnormal brain development. Recent studies have shown that endosymbiotic bacteria of mosquitoes (*Wolbachia*) can block ZIKV transmission[1]. *Wolbachia* alters the profile of host microRNAs (miRNAs), suggesting a potential mechanism for interfering with ZIKV. We predict that *Wolbachia*-dependent mosquito miRNAs may directly interact with viral RNA (vRNA) or indirectly suppress ZIKV. This study can help us gain a deeper understanding of the mechanisms underlying the blockade of virus transmission.

How does *Wolbachia* suppress ZIKV?



Could small RNAs play a role in ZIKV Interference?

Mosquito miRNAs are differentially-expressed in response to *Wolbachia* presence.



Objective

We are exploring the *Wolbachia*-mediated host miRNA suppression on ZIKV load in mosquito C6/36 cells.

Methods

Workflow

Literature Review:
find mosquito miRNAs for which expression depends on *Wolbachia* and/or ZIKV infection

Predict miRNA-vRNA pairs:
select host miRNAs that bind with viral RNA using miRNA prediction tool miRanda [2]

Mimicking *Wolbachia* effects
in mosquito cells:
measure the ability of selected miRNA in reducing ZIKV in C6/36 (W-) cells

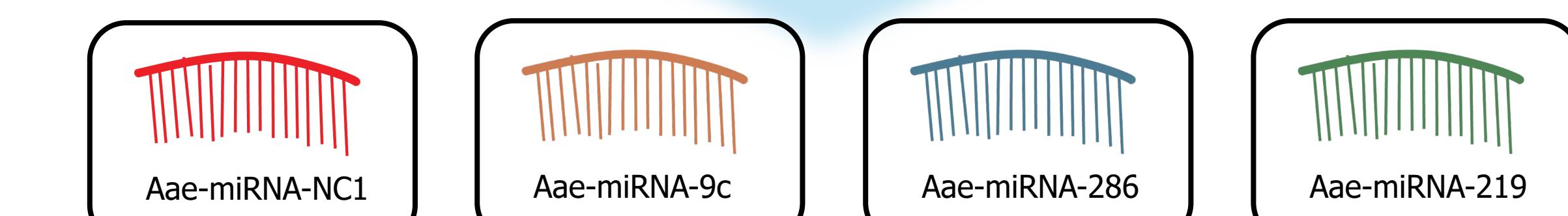
Mimicking *Wolbachia* effects
in mammalian cells:
measure the ability of selected miRNA in reducing ZIKV in Vero cells.

Literature Review

Table 1. Review of differentially-expressed mosquito miRNAs in response to *Wolbachia* and/or ZIKV-infection.

Aae-miRNA	Change of expression in Response to <i>Wolbachia</i>	Change of expression in Response to ZIKV	miRNA-mRNA pair
219	Overexpressed in <i>Ae. aegypti</i> Aag2 cells [3]	No Information	Predicted
286	Overexpressed in <i>Ae. aegypti</i> Aeg2 cells [4]	Reduced at 2, 7, and 14 days after ZIKV infection [5,6]	Predicted
9c	Overexpressed in <i>Ae. aegypti</i> Aeg2 cells [3]	No Information	Predicted

Approach



Analysis

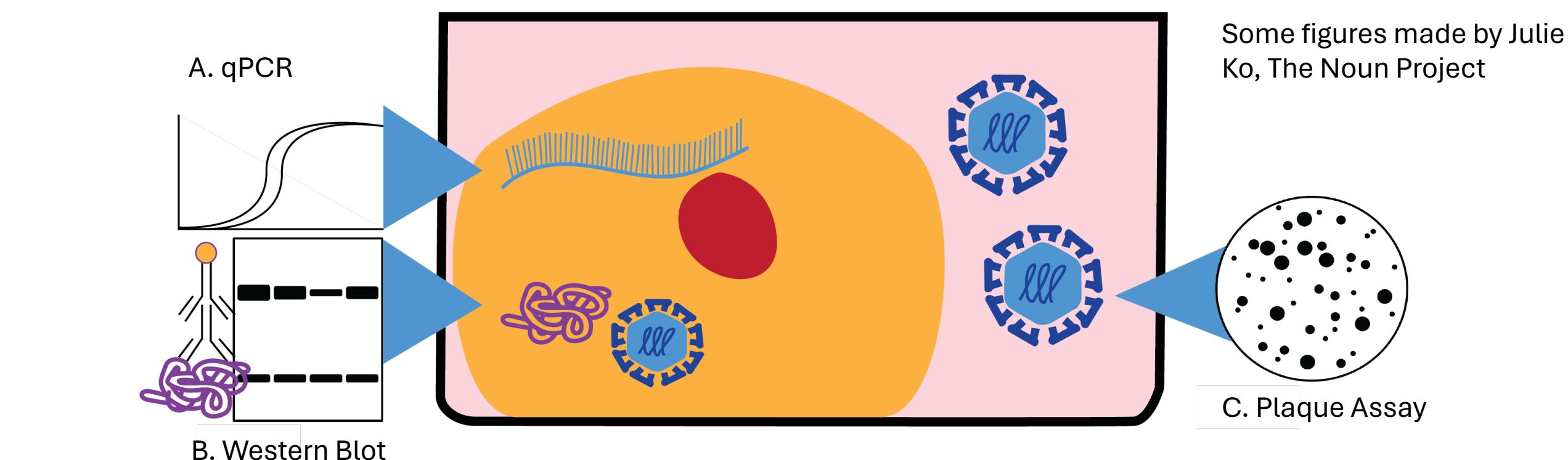


Figure 1. We introduced *Ae. aegypti* miRNA: 9c, 286, 219, and negative control (miR-NC) into C6/36 cells. To test effects of these miRNAs on ZIKV, we use (A) qPCR to measure viral RNA copy number and (B) Western blots, measure the amount of viral proteins inside the cell, (C) plaque assay, looking at viral titers outside of the cell.

Results

Do selected mosquito miRNAs suppress ZIKV in mosquito cells?

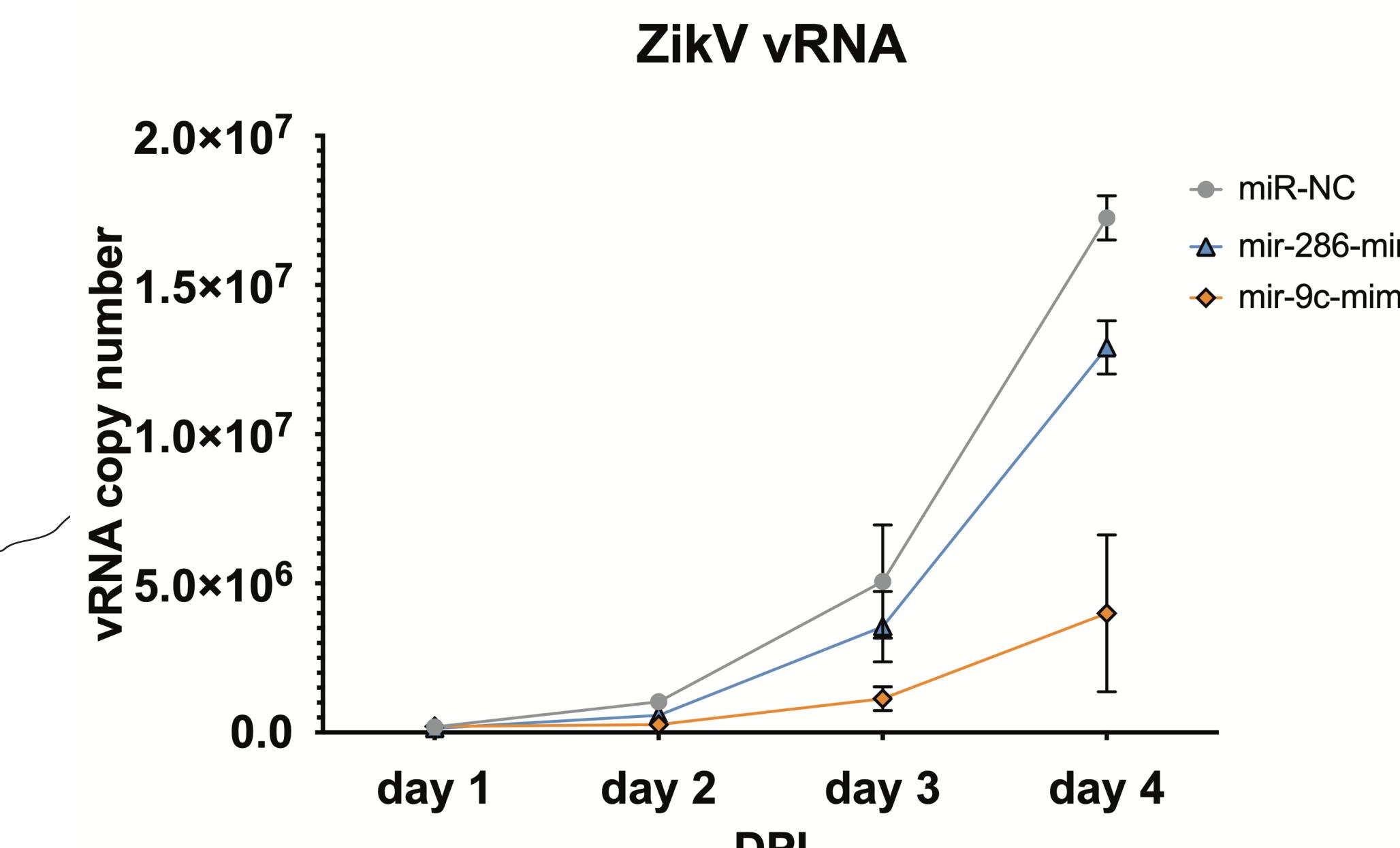


Figure 2. ZIKV vRNA copy numbers in response to miRNA treatment in C6/36 cells. vRNA copy number in response to aae-miRNA-9c and aae-miRNA-286 treatments from 1 to 4 days post infection (DPI). Both aae-miRNA-9c and aae-miRNA-286 show a reduction in comparison to the control miR-NC. The aae-miRNA-9c has the strongest reduction of ZIKV.

Future Directions

- Test mosquito miRNAs in ZIKV-infected Vero cells
- Measure the suppression of ZIKV in placental cell lines
- Explore the roles of other *Wolbachia*-dependent *Aedes* miRNAs in ZIKV suppression

[1] Dutra, HL et al. 2016 *Cell Host Microbe*

[2] Stark, A. et al. 2003 *PLoS Biol*

[3] Mayoral, JG et al. 2014 *PLoS ONE*

[4] Bishop, C. et al 2022 *Sci Rep*

[5] Yen, P. S. et al. 2019 *Viruses*

[6] Saldana, M. A et al. 2017 *PLoS Negl Trop Dis*