

Expression of TaV Tagged Sindbis Virus (TR339) in *Aedes albopictus* Cell Lines and Adult Mosquitoes

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Arthropod-borne-viruses (arboviruses) pose a global threat due to their ability to be transmitted by hematophagous insects to vertebrate hosts resulting in a range of serious infectious diseases [1]. Sindbis virus (SINV) is an arbovirus of the genus *Alphavirus* and the family *Togaviridae*. This virus is imbibed during bloodfeeding by female mosquitoes in order to enrich their egg production with protein and cholesterol [2]. Bloodmeals are deposited in the mosquito midgut and virions must first cross the gut ('gut barrier') via infection to enter the hemolymph and contact various target tissues [3,4]. The mosquito midgut is a simple columnar epithelium surrounded by a basement membrane, peristaltic muscle bundles, nerve fibers, and tracheoles [5].

For a permissive infection route, the virus penetrates the luminal apical aspect, the interface between the bloodmeal and the midgut cells. The bloodmeal typically has two barriers; first is a peritrophic matrix that prevents direct contact of the bloodmeal with the epithelial layer [6] and second the columnar cells that makeup the midgut are covered with microvilli including a complex microvilli-associated network [7].

The purpose of this study was to investigate the use of a tagged virion in the *in vitro* and *in vivo* environments. The fluorescent protein GFP was inserted between the Capsid and PE2 in the genome of TR339; SINVTaV-GFP [8]. During virus translation, GFP is attached to PE2, a precursor protein that gets differentially cleaved in the cytoplasm. This virus construct then has the same infectivity and virulence as wild type TR339 [8], and will leave a fluorescent 'path' in infected cells revealing the virus transit.

This study used two *Aedes albopictus* mosquito cell lines, C7-10 and C6/36, to monitor the infection of stock virus compared with progeny virus. The C7-10 cells were first infected with SINVTaV-GFP and the supernatant (progeny virus) was collected. Both SINVTaV-GFP and progeny were used to infect C7-10 and C6/36 cells in glass cover-slipped chambers. Images were taken at various time points post-infection (p.i.). Presence of GFP was observed at 6 hours p.i. with confluent presentation at 24 hours p.i. in stock and progeny virus, showing good retention of reporter protein.

We determined that fluorescent quenching and cell leakage of proteins was not observed. Fluorescent quenching was assayed following two challenges; a 24 hour time period with consistent beam exposure and 2 weeks on a countertop exposed to direct sunlight. Secondly cells were fixed with 4% paraformaldehyde in 0.1 M Na Cacodylate buffer (pH 7.4) and imaged at 72 hours for any evidence of cell leakage of fluorescence.

Ultimately, *Ae. aegypti* mosquitoes were challenged with a viremic bloodmeal at a titer of 10^7 PFU/ml SINVTaV-GFP and midguts were dissected over several days. The presence of GFP was observed at day 3 p.i. as a small foci and day 5 p.i. as a larger foci area. The GFP was observed in the columnar epithelia cells at these time points supporting gut infection [9].

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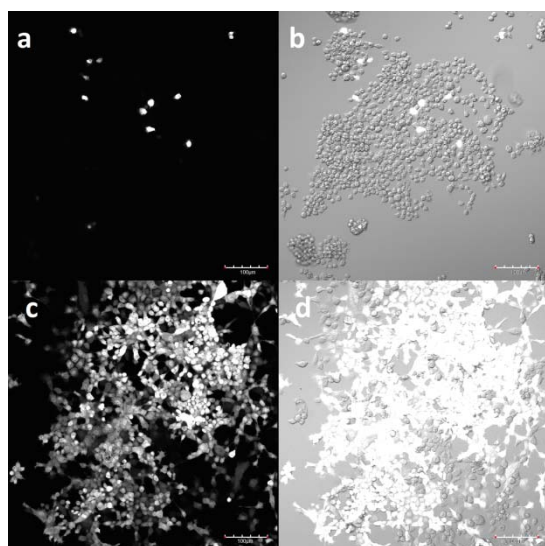


Figure 1. Fluorescent confocal images of C7-10 cells infected with SINVTaV-GFP at 12 hr (a) and 48 hr (c) p.i. and overlay of DIC images (b) and (d), respectively. 100 micron scale bar.

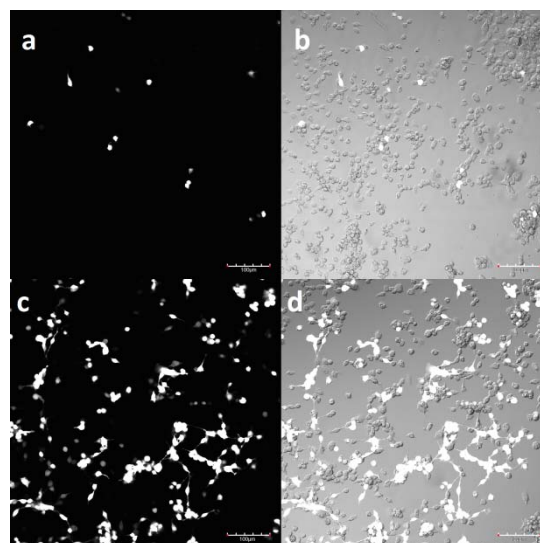


Figure 2. Fluorescent confocal images of C6/36 cells infected with SINVTaV-GFP at 12 hr (a) and 48 hr (c) p.i. and overlay of DIC images (b) and (d), respectively. 100 micron scale bar.

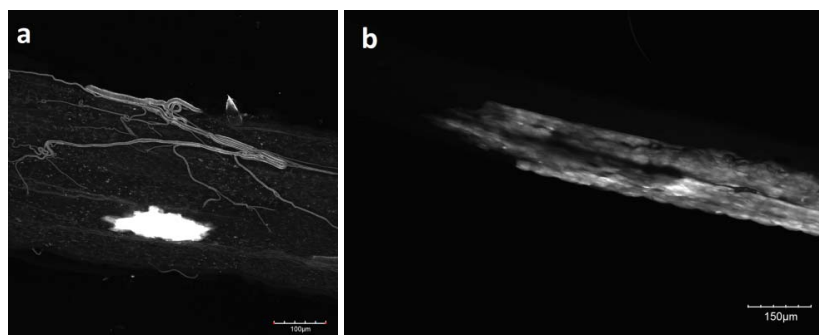


Figure 3. Fluorescent confocal images of *Ae. aegypti* midguts infected with SINVTaV-GFP. (a) Day 3 p.i. 100 micron scale bar and (b) Day 5 p.i. 150 micron scale bar.