**Gypsies, PAMPs and IIPs: three projects using a biochemical lens to study how mosquito-borne viruses are transmitted and emerge into human populations**

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1. **Gypsies: How do mosquito-borne viruses use gypsy transposons encoded by the mosquito genome to replicate in mosquito cells to enable viral transmission?**

Arthropod-borne viruses (arboviruses) transmitted by the ‘yellow fever mosquito’ *Aedes aegypti*, including dengue, Zika, yellow fever, and chikungunya viruses, are causing an ever-increasing disease burden around the world. A better understanding of how arboviruses interact with *Ae. aegypti* at the molecular level would allow us to design new approaches to block disease transmission. Approximately 50% of the *Ae. aegypti* genome is made up of transposons, which are mobile genetic elements that can ‘jump’ (transpose) within their host’s genome. Transposons have been implicated in a number of cellular and disease processes, including immunity, placental development, and cancer. Recently, a specific group of transposons called LTR retrotransposons was shown to be critically important for arbovirus replication in mosquitoes. While we know that these transposons are therefore important for arbovirus transmission, whether their expression is altered during arbovirus infection remains unknown. In this project you will study how LTR retrotransposon activity is affected by arboviruses using a fluorescent reporter construct that allows us to measure the activity of one specific LTR retrotransposon called ‘gypsy’. When gypsy is active in a mosquito cell, red and green fluorescent proteins are expressed that turn the nucleus red and the cell membrane green (hence the reporter is called a ‘Watermelon’ reporter).

**Project Aims:**

1. Identify conditions that cause gypsy to activate. This will involve testing different mechanisms of inducing cellular stress, including heat shock and drug treatments.
2. Test how the individual expression of different dengue virus proteins in mosquito cells changes the activation of gypsy when activated using your conditions identified in (1).
3. Test whether individual expression of different dengue virus proteins in mosquito cells can itself activate gypsy.

Overall, this information will tell us how dengue virus affects gypsy activity to allow the virus to replicate in mosquito cells and eventually be transmitted to humans.

**Key skills learnt:**

You will learn how to culture eukaryotic cells, how to treat cells to induce cellular stress, and how to transfect cells with plasmids (including our gypsy Watermelon reporter and individual dengue virus proteins). You will learn how to visualise protein expression by western blot, and will use fluorescence microscopy and flow cytometry to measure gypsy and viral proteins.

**References:**

Goic, B. *et al.* (2016) Virus-derived DNA drives mosquito vector tolerance to arboviral infection. *Nature Communications.* 7:12410 (<https://www.ncbi.nlm.nih.gov/pubmed/?term=27580708>)

Chang, Y. *et al.* (2018) Cellular labelling of endogenous virus replication (CLEVR) reveals de novo insertions of the gypsy endogenous retrovirus in cell culture and in both neurons and glial cells of aging fruit flies. *BioRxyv* <http://dx.doi.org/10.1101/445221>

1. **PAMPs: How do mosquito cells detect viral pathogen-associated molecular patterns (PAMPs) to block mosquito-borne virus transmission?**

The mosquito *Aedes aegypti*, which transmits dengue, Zika, yellow fever, and chikungunya viruses, is a useful model for other arthropod-borne (arboviruses) of human and veterinary importance due to the genetic and molecular tools available. The transmission dynamics and global emergence of arboviruses are driven in part by changing virus-host interactions in the mosquito vector. Mosquito immune responses pose strong barriers to virus transmission, and shape the evolutionary landscape of arbovirus emergence. The NF-κB immune signalling pathways are conserved from invertebrates to vertebrates, but how these pathways detect and block virus infection in mosquitoes remains poorly understood. With a better understanding of how mosquito cells detect incoming viral infection, we might be able to engineer mosquitoes that are better able to fight of arbovirus infection to prevent disease transmission to humans. We showed for the first time that viral RNA is a strong PAMP that is sensed by mosquito cells to activate an NF-κB pathway called ‘IMD’ (which is similar to the human TNF signalling pathway). We hypothesise that this viral RNA must be sensed by an RNA-binding protein that acts as a pattern recognition receptor (PRR) encoded by the mosquito genome, and this project will try and identify this PRR protein.

**Project Aims:**

1. Identify which RNA-binding proteins encoded by the mosquito genome can sense viral RNA by silencing their expression and testing the effect this has on the ability of viral RNA to stimulate the IMD pathway.
2. Clone the PRR you identify in (1) and test whether it colocalises with viral RNA in cells treated with viral RNA, or cells infected with virus.
3. Test whether your cloned PRR interacts directly with viral RNA using biochemical assays such as EMSA (electrophoretic mobility shift assay).

Overall, this information will tell us which RNA-binding proteins encoded by the mosquito genome act as PRRs to detect viral infection to prevent the transmission of arboviruses to humans.

**Key skills learnt:**

You will learn how to culture eukaryotic cells, how to treat cells with RNA to activate immune signalling, and how to transiently transfect cells to express proteins or RNAs. You will learn how to prepare your own double-stranded RNAs (dsRNAs) for silencing gene expression in insect cells using RT-PCR and *in vitro* transcription. You will learn how to measure immune activation using luciferase reporter assays and/or RT-qPCR. You will also use immunofluorescence microscopy to measure the colocalization of RNA and proteins in cells, and will use EMSA to test protein-RNA interactions.

**References:**

Merkling, S. & van Rij, R. (2013) Beyond RNAi: antiviral defense strategies in Drosophila and mosquito. *Journal of Insect Physiology*. 59:159-170 (<https://www.ncbi.nlm.nih.gov/pubmed/?term=22824741>)

1. **IIPs: How does mosquito-borne virus emergence correlate with the ability of viral immune inhibitor proteins (IIPs) to block NF-κB responses in mosquito cells?**

Viruses are in an evolutionary arms race with their hosts. The host is continually evolving new ways for the immune system to detect incoming viral infections. Meanwhile, viruses are constantly evolving new ways to hide from or block the antiviral immune systems of the host using IIPs. As a result of this arms race, viruses are highly specialised to the host species they infect. Thus, arthropod-borne viruses (arboviruses) encode IIPs that allow them to escape from immune clearance in a limited range of mosquito species, which restricts the range of mosquitoes that arboviruses can be transmitted by. To emerge as human pathogens, arboviruses therefore must evolve IIPs that can block the immune system of those specific mosquito species that feed on humans, such as *Aedes aegypti*. A better understanding of how viruses evolve IIPs to escape immune responses to enable replication in the right species of mosquitoes for human transmission might help us to predict which arboviruses might emerge as new diseases of human public health importance. We recently showed that dengue virus serotype 2 (DENV-2), a highly successful human pathogen transmitted by *Ae. aegypti*, blocks the activation of a mosquito NF-κB pathway called ‘IMD’ (which is similar to the human TNF signalling pathway) in *Ae. aegypti* cells through the action of the viral NS4A protein. We hypothesise that other DENV serotypes (DENV-1, -3, -4) and related flaviviruses transmitted by *Ae. aegypti* (e.g. Zika virus and yellow fever virus) should all be able to similarly block the *Ae. aegypti* IMD pathway. In contrast, viruses that are not transmitted by *Ae. aegypti*, such as Japanese encephalitis virus (transmitted by *Culex* mosquitoes), tick-borne encephalitis virus (transmitted by ticks) or Modoc virus (transmitted without the help of a vector), should not be able to block the *Ae. aegypti* IMD pathway.

**Project Aims:**

1. Clone the NS4A proteins of diverse flaviviruses transmitted through different routes (*Aedes* mosquitoes, non-*Aedes* mosquitoes, ticks, non-vector transmission).
2. Test the ability of these cloned NS4A proteins to block the activation of the *Ae. aegypti* IMD immune pathway by viral and other immune stimuli.
3. Test whether the ability of these NS4A proteins to block immune activation correlates with their ability to localise correctly in the cell and bind their target host proteins.

Overall, this information will tell us whether the IMD immune pathway is part of the evolutionary arms race that can explain how arboviruses emerge to cause human disease through adaptation to replicate in human-biting mosquitoes.

**Key skills learnt:**

You will learn how to culture eukaryotic cells, how to treat cells to activate immune signalling, and how to transiently transfect cells to express proteins. You will learn how to measure immune activation using luciferase reporter assays and/or RT-qPCR. You will also use immunofluorescence microscopy to measure the localization of proteins in cells, and will use immunoprecipitation and western blots to study protein-protein interactions. You will also clone your own viral protein expression plasmids.

**References:**

Mitchell, P. *et al.* (2012) Evolution-guided identification of antiviral specificity determinants in the broadly acting interferon-induced innate immunity factor MxA. *Cell Host Microbe* 18:598-604 (<https://www.ncbi.nlm.nih.gov/pubmed/23084925>)

Merkling, S. & van Rij, R. (2013) Beyond RNAi: antiviral defense strategies in Drosophila and mosquito. *Journal of Insect Physiology*. 59:159-170 (<https://www.ncbi.nlm.nih.gov/pubmed/?term=22824741>)