Investigating localization of RNAs from the Tick Borne Virus (TBEV) in infections of mammalian cells via FLIM-Riboglow

GEORGETOWN UNIVERSITY

Marie Abboud, Emilia Moscoso, Esther Braselmann

Department of Chemistry, Georgetown University, Washington, District of Columbia, USA

Abstract

RNAs play key roles in fundamental biochemical processes and their dynamics are associated with many disease states, motivating the need to visualize RNAs live. For instance, flaviviruses are RNA viruses that infect over 50 million people annually. A variety of methods have been developed to track RNAs. The MS2-GFP system utilizes a fluorescent protein fused to a modified MS2 protein. The Braselmann lab has developed Riboglow-FLIM, a fluorescent lifetime-dependent RNA tagging platform. The end goal of this project is to design a Riboglow-tagged viral system to image live virus-host interactions during TBEV infection, ultimately gaining a better understanding of flaviviruses in general.

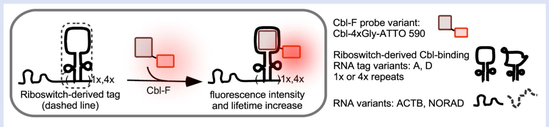

FIgure 1. Riboglow-FLIM provides a mechanisms for tracking RNA in live cells. A Cobalamin (Cbl) fluorophore probe experiences an increase in fluorescence intensity and lifetime when it binds to a specific RNA sequence. This change in fluorescence and lifetime can be tracked using fluorescence lifetime imaging microscopy (FLIM) (Sarfraz et al. Nat.Commun 14,867 (2023).

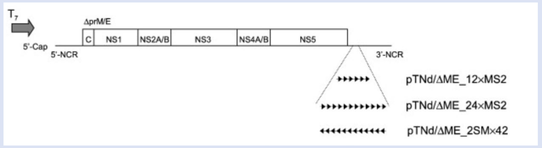

Figure 2. In the MS2 system, non structural protein genome of the tick-borne encephalitis virus (TBEV) is modified to contain 24 repeats of the MS2 aptamer. Additionally, a green fluorescent protein (GFP) is fused to the MS2 aptamer. The MS2-GFP reveals the location of TBEV in live cells (Miorin, L et al. Virology 64-77 379 (1) 2008).

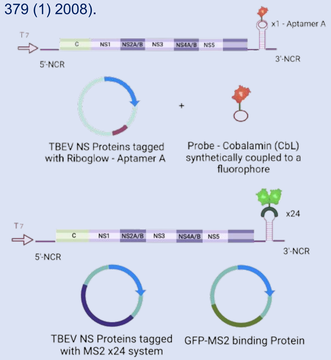

Figure 3. Riboglow-FLIM system. Cobalamin fluorophore probe fluoresces when it binds to riboswitch RNA.

Figure 4. MS2-GFP system. Green fluorescent protein binds to MS2 aptamer. This system attaches to viral RNA and fluoresces, making is possible to track viral RNA in live cells.

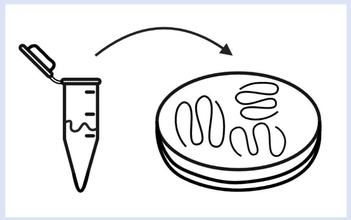

Figure 1. Schematic representation of a transformation. DNA plasmid of interest is introduced into plates containing ampicillin resistant bacteria, where it will be stored and replicated.

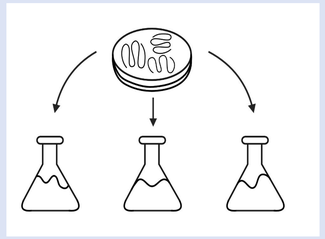

Figure 2. Representation of an overnight bacterial culture. Bacterial colony containing DNA plasmid of interest is introduced into flasks with LB broth for overnight incubation.

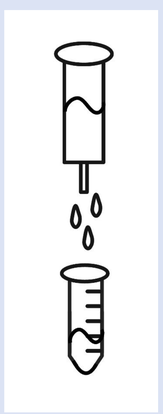

Figure 3. Schematic representation of a midiprep. DNA produced during overnight is purified. This is done by isolating replicated DNA from bacteria. Purified DNA drips into conical tubes, where it can be stored for transfection.

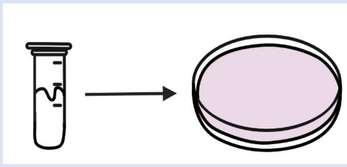

Figure 4. Representation of the transfection process. DNA of interest is transfected into HeLa cells. Cells will replicate the DNA through translation.

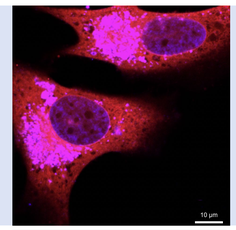

Figure 5. FLIM image of live HeLa cell transiently transfected with mScarlet, which localizes in the ER, and mTurquoise, which localizes in the golgi. Cells stained with blue nuclear dye.

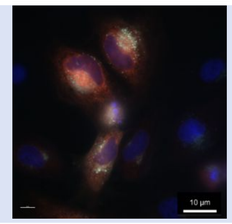

Figure 6. Image of HeLa cell taken with Nikon microscope. Cells transiently transfected to produce mScarlet, localized in the ER, and mTurquoise, localized in golgi. Cells stained with blue nuclear dye.

FLIM: Fluorescent Proteins

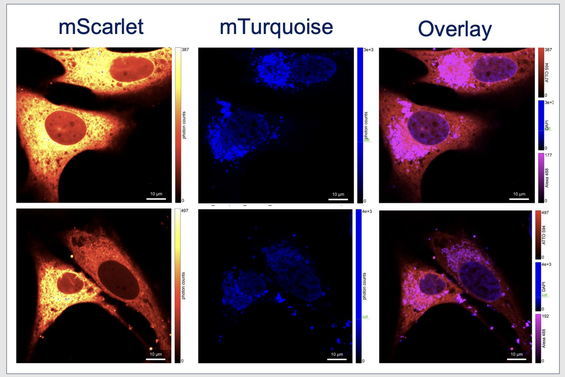

Figure 5. Live HeLa cells transiently transfected with mScarlet and mTurquoise and stained with blue nuclear dye. Imaged with FLIM microscope. Leftmost slide was imaged with ATTO 594 laser; shows mScarlet localized in ER. Middle slide was imaged with DAPI laser; shows mTurquoise localized in golgi. Rightmost image shows overlay of the other two images.

ECLIPSE Ti2 inverted microscope: ΔPTND-24xMS2

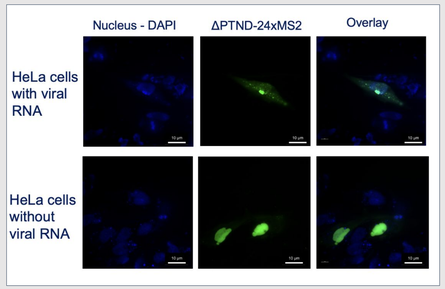

Figure 6. HeLa cells transfected with MS2-GFP system and stained with blue nuclear dye. Imaged 24 hours after transfection with Nikon microscope. Leftmost slides imaged with DAPI laser, and middle slide imaged with CFP laser. All images taken at 100x. As expected, MS2-GFP system was located in the nucleus when the cell did not contain viral RNA. When there was viral RNA, the MS2-GFP system was located outside the nucleus, in the ER.

ECLIPSE Ti2 inverted microscope: Fluorescent Proteins

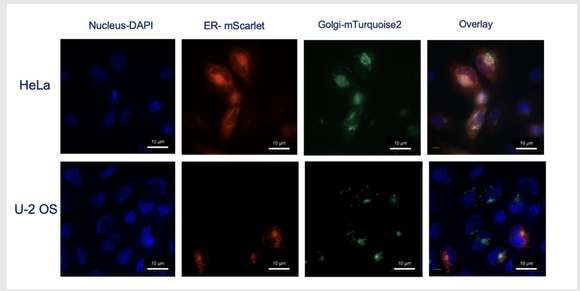

Figure 7. HeLa and U-2 OS cells stained with blue nuclear dye and transfected with mScarlet and mTurquoise. Imaged with Nikon microscope. Leftmost slide imaged with DAPI laser at 365 nm; shows cell nucleus. Middle two slides were imaged with with lasers at 594 nm and 488 nm, respectively. Rightmost image shows overlay.

ECLIPSE Ti2 inverted microscope: ΔPTND-24xMS2

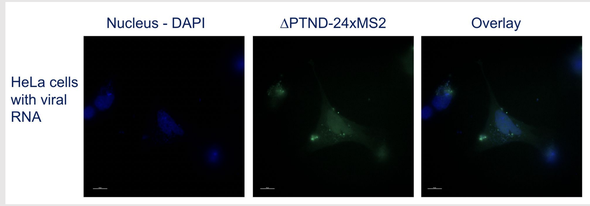

Figure 6. HeLa cells transfected with MS2-GFP system and stained with blue nuclear dye. Imaged 48 hours after transfection with Nikon microscope. Leftmost slide was imaged with DAPI laser at 365 nm, and middle slide was imaged with CFP laser at 440 nm. All images were taken at 100x. NA= 1.45 and WD=130 µm. As expected, MS2-GFP system was located outside of the nucleus, in the ER, when viral RNA was present.

Conclusions

- Successfully monitored co-localization between ER and Golgi fluorescent protein markers for future confirmation of replicated TBEV RNA localization
- Designed first viral Riboglow-FLIM tracking system
- Presents the opportunity for future research involving kinetic analysis of viral replication

Next steps

- Reproduce time lapse figure with MS2 system in U-2 OS and HELA cells
- Monitor the subcellular localization of replicated RNA in U-2 OS and HeLa cells using Riboglow-FLIM - TBEV

Acknowledgements

Special thanks to Emilia Moscoso, Harrison Lee, Esther Braselmann and all the members of the Braselmann lab. Additionally, thanks to Colleen Dougherty and the Center for Research and Fellowships for their help this summer. Finally, thanks to the Laidlaw Foundation for their generous support.

References

1. Braselmann, E *et al. Nat. Chem. Biol.* 14, 964 (2018)
2. Sarfraz et al. Nat.Commun 14,867 (2023)
3. Miorin, L et al. Virology 64-77 379 (1) 2008