November 2022 Issue No. 8

Colorado RNA Club

LATEST

This month... Viral RNAs

What's new from our RNA community

Letter from the editor

Viruses are fascinating. They are simply genetic material enclosed in a protein coat. Despite this, viruses infect hosts, evade their immune system, and replicate, sometimes to devastating effect.

Nothing exemplifies this more than SARS-Cov-2 that has inundated our lives for the past few years and in the process infected ~630 million and killed ~6.6 million people, globally. However, it is worth noting that there are other viruses, which are or have the potential to be just as deadly.

In this issue of the Colorado RNA Club Latest we highlight ongoing research on other viruses, namely flaviviruses. We feature Tim Stasevich and Brian Geiss who, in collaboration, are investigating flaviviral gene regulation by imaging their translation; David Beckham who is using different lines of investigation to develop vaccines for these viruses; and James Burke, an alumnus of the Parker lab who discovered a mechanism by which viruses, including flaviviruses, can reprogram cellular processes like RNA decay.

We also have some updates from *Latest*. This newsletter will now be bi-annual. We have a whole new team of volunteers joining us. Kate Segar, Mlana Lore, Elizabeth Murphy, and Gaia Bublitz have joined us as interviewers/writers, and Gabriel Galindo has taken over as designer.

Finally, after two years in the role, this will be my last issue as editor; I'm handing over my role to Kate Segar and Mlana Lore. It has been an absolute privilege to work on the newsletter team for the past three years, and I look forward to the exciting issues ahead.

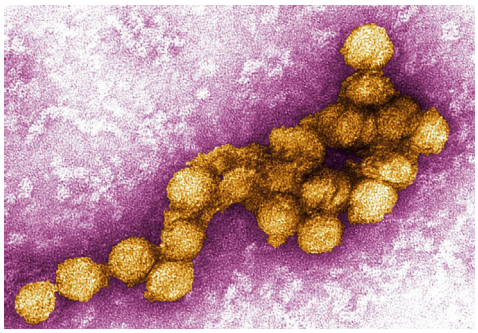
As always, we hope you enjoy reading this issue.

Divya Kolakada

Editor, Colorado RNA Club Latest

Imaging Translation in Search of the Achilles Heel of Viruses

by: Libby Murphy



West Nile Virus, *NIH*

If we've learned anything from the last few years, it's that viruses play a pivotal role in a host of biological systems. Tim Stasevich, Ph.D. and Brian Geiss, Ph.D., are working to learn more about the gene regulation tricks viruses have developed over the eons, hoping to open doors in virology and cellular translation dynamics as well as in the clinic.

The Stasevich and Geiss labs are using the Stasevich lab's novel technique called Tethering 'n' Translation (TnT) in combination with the Geiss lab's viral reagents and expertise to dig into flaviviruses' gene regulation secrets. Tim was motivated to study this topic to advance our ability to control cellular translation dynamics. "I have been interested in imaging viral RNA translation [ever since] we created our technology to image mRNA translation more generally," he says.

We featured TnT in a previous article—so how's it going in practice?

Brian is encouraged by the novel insights they've gained into flavivirus biology. He describes observing "translation occurring earlier than [they] previously thought it would, viral polyprotein translation turning on and off during infections, and intracellular spread of viral polyprotein translation sites that are demonstrating multiple rounds of translation are occurring during infections." Interestingly, they've also noticed that translation sites tend to switch off after some time, with new ones appearing later. They

hypothesize that this is the viral genome transitioning between translation and replication states. Tim also suggests the possibility that "viral translation sites are being actively targeted by anti-viral machinery".

"Either way, it'll be really exciting to better characterize these kinetics," says Tim.

Tim is particularly excited about the "robust, rapid response" they've observed by using viral reporters. "I was thrilled to see hundreds of tiny translation sites just minutes after we infected cells with our West Nile Virus (WNV) replicon. The translation sites were bright and easy to track, and the majority were localized to the endoplasmic reticulum where translation was expected to occur."

The findings that Tim's and Brian's labs are making hold promise for further discovery as well as future vaccines and therapeutics. Tim dreams of finding an "Achilles heel" which could be pivotal in future therapeutics against viral infections. He adds, "the ability to see viral translation is just plain cool. Imaging this very early step in viral lifecycles had not been possible before, so everything we see will be a 'world first.'" Brian is just as enthusiastic about the future promise of this technology. "Being able to visualize translation of viral polyproteins in live cells opens up quite a few different avenues for research as well as for therapeutic and vaccine development," he says. "Understanding where and when these proteins are translated in infected cells provides crucial information that we can use to help develop better vaccines and antivirals against these pathogens". With so many possibilities for how his and the Stasevich lab's work can advance science and medicine, Brian adds that "the challenge is really choosing what we want to study!"

Alumni Spotlight: James Burke

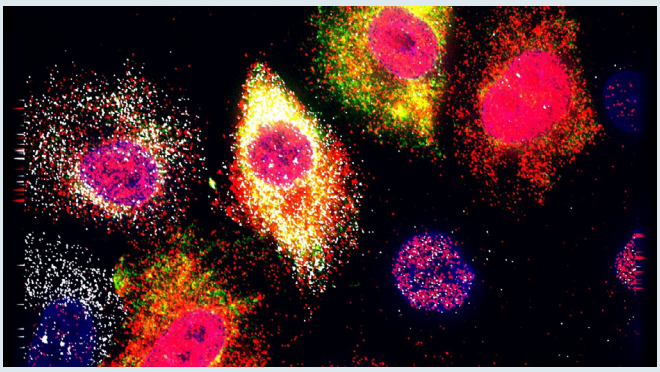
Viruses: Master Manipulators of RNA Biology and the Immune System

by: Mlana Lore

James Burke, Ph.D.,
Personal Photo Collection

In this alumni spotlight, I spoke with James Burke, Ph.D., a former postdoctoral fellow in Roy Parker's lab at CU Boulder. He is now an Assistant Professor at the University of Florida (UF) Scripps Biomedical Research Center, taking advantage of the recent merger between UF and the Jupiter campus of the Scripps Research Institute. As a new PI, he is building a lab focused on understanding the interface of RNA biology, immunology, and virology and applying this knowledge to translational research.

Burke's fascination with RNA biology and viruses has remained steady throughout his career. He came to Colorado in search of cutting edge research after graduating from UT Austin, where he studied DNA tumor viruses and viral miRNAs in the Sullivan lab. Burke was drawn to Roy Parker's lab to answer fundamental questions about RNA biology and virology. During his postdoc, Burke discovered unexpected and contradictory ways in which viruses use host antiviral defenses to their advantage. We often think of viruses as hijacking the host cell replication machinery. But, Burke explained, they are actually doing more complicated cellular biology than was previously appreciated, and are capable of completely reprogramming cellular processes like RNA decay, translation, and innate immunity. One striking example is the relationship between viruses and host RNase L. It was previously thought that RNase L was activated by host cells to degrade viral RNAs, inhibiting translation and viral replication. Surprisingly, Burke found that RNase L could also influence viral replication and the innate immune response through complex and novel mechanisms including regulating differential mRNA decay and ribonucleoprotein complex formation, blocking mRNA export, and modulating the type I interferon response. In light of the COVID-19 pandemic and recent spotlight on mRNA vaccine technology, Burke stresses the importance of training young virologists to



Influenza A virus infected cells, *Courtesy of James Burke*

rigorously investigate these previously overlooked complexities of viral infection and RNA biology.

Burke looks back fondly on his time studying RNA in CO saying, “you can’t really be in a better place, so it’s hard leaving the community”. He hopes to bring the spirit and collaborative nature of the CU RNA community with him to UF. Burke always knew he wanted to be a PI so he would have the freedom to investigate the questions that were the most exciting to him. Right now those questions center around understanding how viruses manipulate host cells to regulate the RNA life cycle and the innate immune system to their advantage. As master manipulators of host defenses, certain viruses, including SARS-CoV2, Dengue, and Zika, have evolved to escape RNase L-mediated RNA decay. This is where Burke plans to employ the drug screening capabilities of UF Scripps, to search for ways to specifically target RNase L to viral RNAs and, he says excitedly, “to push [some] out-there ideas”. Despite his lengthy publication list and success in academia, Burke emphasizes the importance of work-life balance in doing great science, reiterating his undergraduate PI’s advice to “live life and just do science while you live life”.

Outmaneuvering the Flavivirus

by: Kate Segar

The Beckham Lab at CU Anschutz houses scientists who are keen to understand the “crosstalk between immunity, RNA viruses, and the brain”, with the aim of translating their discoveries into potential therapeutics. David Beckham, M.D., has a keen interest in flaviviruses: a family of pathogenic, mosquito-borne RNA viruses that include the West Nile, Dengue, and Zika viruses.

One intriguing aspect of these viral RNAs is their structure. Although they have a typical 5’ cap, they have a highly structured 3’UTR instead of a poly(A) tail.

In 2015, the lab began a collaboration with the Kieft Lab to understand the function of pathogenic flaviviral 3’UTRs using RNA structure as a guide. The complex structure of these 3’UTRs make them resistant to the ribonuclease XRN1. Thus, they are often incompletely digested and form non-coding RNAs termed subgenomic flaviviral RNAs. The Beckham lab uses structural insights from the Kieft lab to make specific mutations that disrupt viral structures in their clone-derived virus model. They have found success with some of these mutations which disrupt the ability of the viruses to make sfRNAs, resulting “in a kind of disease attenuated phenotype where the virus is still able to replicate but it just doesn’t cause disease.”

“That’s the ideal vaccine in an immune-competent person,” Beckham asserts.

In another ongoing project, the lab focuses on developing a vaccine for Dengue. Dengue affects millions of people every year, most often children. It has four serotypes which are essentially four different viruses that are closely related. This makes developing a vaccine for the virus a challenge: “The problem with

The Beckham lab, *Personal Photo Collection*

Dengue is that [your vaccine has to] have really strong antibody and immune responses to all four serotypes. If you make a good immune response to one but not to the other, then you actually enhance disease when a person is exposed to another serotype,” Beckam explains. The Beckham lab’s approach to this issue is to make a chimeric RNA molecule that incorporates components of each Dengue serotype to prime the immune system equally against each serotype. It is a challenging undertaking but the lab has preliminary results that show promise.

While Beckham was initially drawn to research as a means of discovery for therapeutics, his favorite part of his job now are the people he has populated his lab with. “The students are by far the best part about being a PI. It’s so much fun to see them succeed and move forward with their careers. When they leave the lab and find success in their career, it’s really gratifying.”

Announcements

Seminars & Talks

Ralph Kleiner, Ph.D

November 9, 2022 | 3:30pm-4:45pm | In-person/A115 Butcher Auditorium | CU Boulder

Lab website: https://kleiner.princeton.edu

Host: Deborah Wuttke

Link: https://www.colorado.edu/biochemistry/events-0

Yongsheng Shi, Ph.D

January 9, 2023 | 12pm-1pm | Hybrid/RC1 South 9th floor conference room | CU Anschutz

Lab website: https://faculty.sites.uci.edu/shi/

Host: Rui Zhao

Link: https://medschool.cuanschutz.edu/biochemistry/upcoming-events/bmg-seminar-series

Publications

Utilization and Potential of RNA-based Therapies in Cardiovascular Disease

Emma Louise Robinson and J. David Port | CU Anschutz

The current review focuses broadly on the concept of nucleic acid (NA)–based therapies, considering the use of various forms of NAs, including mRNAs, miRNAs, siRNA, and guide RNAs, the latter specifically for the purpose of CRISPR-Cas directed gene editing. We describe the current state-of-the-art of RNA target discovery and development, the status of RNA therapeutics in the context of CVD, and some of the challenges and hurdles to be overcome.

Link: https://www.sciencedirect.com/science/article/pii/S2452302X22000389

Jop Openings

Postdoctoral Fellow | Whiteley Lab | CU Boulder

The Alex Whiteley laboratory in the Biochemistry Department at CU Boulder is hiring a postdoctoral researcher to work on UBQLN2 in ALS-FTD based on our recent BioRXiv paper, which concerns activities of a virus-like, RNA-binding protein which we think contributes to disease. We are particularly interested in candidates with a background in neuroscience or cell biology but encourage all interested to apply!

Email Alex for more information:

alexandra.whiteley@colorado.edu

Link: https://jobs.colorado.edu/jobs/JobDetail/?jobId=41825

Awards & Recognition

Katherine Fantauzzo | Award: R01 | CU Anschutz

Katherine Fantauzzo received a five-year R01 award through the NIDCR titled “Srsf3-mediated alternative RNA splicing in craniofacial development”.

Thomas Forman | Award: F31 | CU Anschutz

MOLB/MSTP student Thomas Forman of the Fantauzzo Lab received a three-year F31 award through the NIDCR titled “Investigating Srsf3-mediated alternative RNA splicing in craniofacial development”

For announcements of recent RNA-relevant seminars, publications, job openings, or awards from your lab, e-mail us at ColoradoRNAclub@gmail.com

Newsletter team
CU Boulder: Gaia Bublitz (Ph.D. student)
CSU: Gabriel Galindo (Ph.D. student)
CU Anschutz: Divya Kolakada (Ph.D. student), Mlana Lore (Ph.D. student), Libby Murphy (Professional Research Assistant), Kate Segar (Ph.D. student)

Supervision: Sujatha Jagannathan (Assistant Professor, CU Anschutz)

Editor: Divya Kolakada Design: Gabriel Galindo