



What's new from our RNA community

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LETTER FROM THE EDITOR

As the common adage goes: change is the only constant.

Scientific techniques are changing at a pace faster than we can keep up with. The evolution of these techniques has transformed the breadth and depth of the questions we can ask; their development is essential to gaining deeper insight into RNA biology. With recently established single-cell and single-molecule techniques for example, we can ask extraordinary questions like how does gene expression vary between different cells within a single tumor? When is a specific factor recruited to a translating mRNA? Where do smaller RNAs like non-coding RNAs localize? Many *Colorado RNA Club* affiliated labs specialize in these novel techniques and in this edition of *Colorado RNA Club's Latest*, we wanted to showcase some of these labs and their work.

We also wanted to share some changes within the newsletter organizing committee. This edition will be the last for some of our editorial team. Laura Baquero, our cartoonist, will be beginning graduate school at the University of Washington this fall. Charlotte Cialek, one of our writers, has completed her thesis defense and will be transitioning to industry soon. Finally, Danielle Bilodeau, editor of this newsletter since its inception last year, is stepping down from her position to prepare for her thesis defense. It is with a touch of sadness that we bid farewell to these members. However, we are excited for them as they begin a new chapter in their lives and wish them all the best in their future endeavors! We also welcome any new interest in volunteering with the editorial team.

I will be stepping in to fill Danielle's shoes as editor. I am excited to take on this new role and I hope to continue her excellent work and leadership.

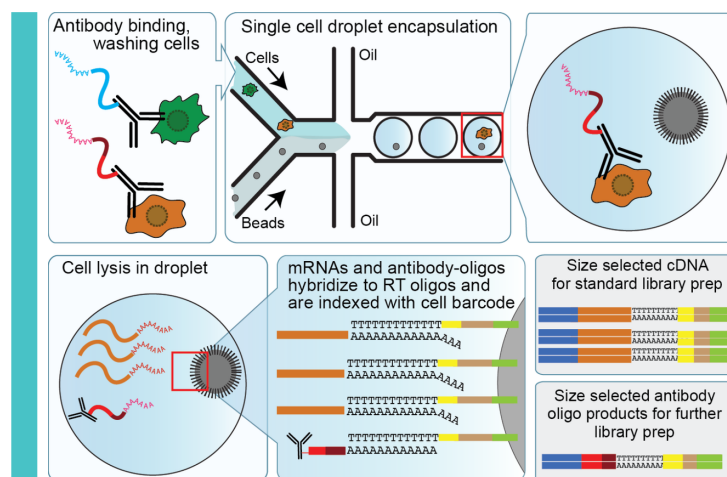
Our team has truly enjoyed compiling this edition for you, and we hope you enjoy reading it.

Divya Kolakada
Editor, *Colorado RNA Club Latest*

Understanding Cells' Heterogeneity

by: Ankita Arora, PhD, Postdoctoral Fellow at CU Anschutz

Cancer development is an evolving process where, in one tumor, cells can be at different stages. Importantly, it is this tumor heterogeneity that often leads to drug-resistant tumors. A better understanding of how tumor cell heterogeneity leads to resistance can expedite the development of alternative therapeutic interventions with improved survival rates.



Schematic of the CITE-seq technology. In short, antibody-bound oligos act as synthetic transcripts that are captured during large-scale oligodT-based scRNA-seq library preparation protocols (e.g. 10x Genomics, Drop-seq). Reference: <https://cite-seq.com/>

The key to understanding the heterogeneous tissue and the dynamic processes within the cells in a single experiment - is the technique called single cell RNA-sequencing (scRNA-seq). Deemed as "Breakthrough of the Year in 2018" by Science, this technique measures the expression of most genes in isolated single cells; thus, allowing for simultaneous profiling of cells based on their individual gene expression characteristics.

The combination of several single-cell technologies will allow scientists to address new questions by deciphering complex mechanisms in heterogeneous samples. Using a variant of scRNA-seq called Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq), the RBI Informatics Fellows, Austin E. Gillen and Rui Fu in collaboration with Shanshan Pei (first author) from the Jordan lab at the Division of Hematology - Anschutz Medical Campus, were able to identify characteristics of patients who respond poorly to venetoclax-based therapy to treat acute myeloid leukemia (AML). This study has additional implications that can facilitate more nuance in the design of new AML therapies.

For example, the study found that the resistant monocytic AML loses expression of the venetoclax target BCL2, and shifts to MCL1 expression for oxidative phosphorylation and survival. Thus, future AML therapies targeting MCL1 inhibition might be a strategy to fight venetoclax resistance. "Analyzing the CITE-Seq data for difference in activated pathways between various populations in the AML patients using a software tool called clustifyr was instrumental for the work", says Rui Fu, PhD.

The RBI Informatics fellows work in collaboration with multiple labs across the campus with the goal to push for large-scale adoption of scRNA sequencing amongst different disciplines. Further, Rui Fu stated that there are three major advancements that the field is progressing towards:

1. Incorporating multiple data-sets or other modal measurements with scRNA data - This becomes especially important for different immune populations which have protein markers that sometimes are missed while looking at RNA dataset alone.

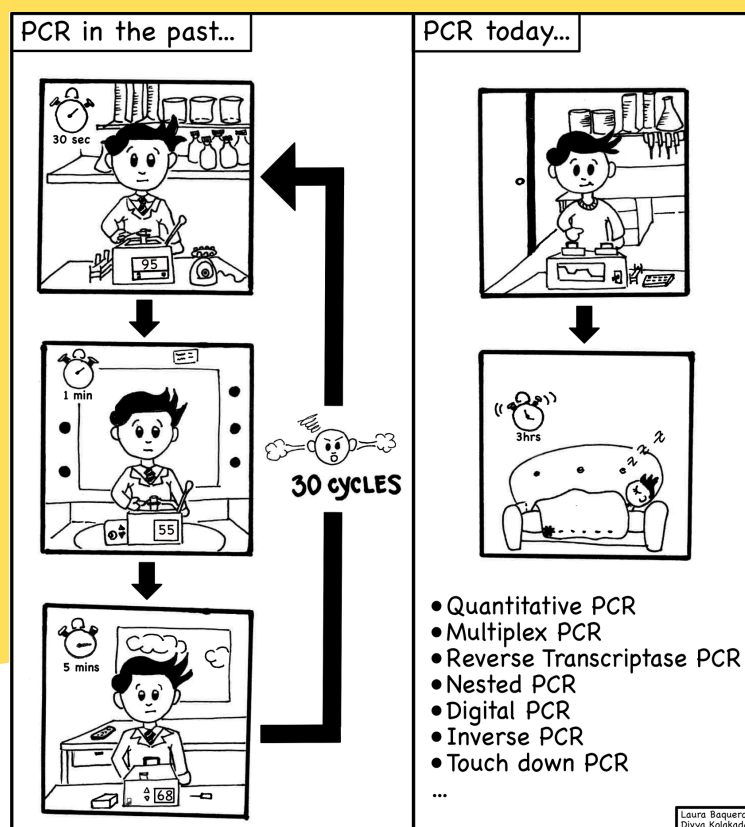
2. Spatial transcriptomics - A profiling method that was named as the Nature Methods of the Year 2020. "Fruit tart is spatial transcriptomics," Bosiljka Tasic, an Allen Institute researcher, said in the Technology Feature by Nature. "You know exactly where each piece of fruit is and what is the relationship of each piece of fruit to the other".

3. Improvising technology, library preparation, and software development in a way that allows for answering mechanistic questions such as RNA decay intermediates and/or alternative polyadenylation sites.

According to Rui Fu, one of the challenges that still remains unsolved is how to strike a balance between how much high-throughput (depth) and how exact information obtained from the scRNA-seq data can be. He says, "Higher gene counts increase the probability to discover more rare populations but that also increases cost and processing time".

Last but not the least, scRNA-seq also offers a one stop-solution for understanding SARS-CoV-2 infection at both levels - firstly monitoring the viral load in the affected cell types and then understanding the landscape of host immune responses *in vivo*.

THE PROGRESSION OF TECHNOLOGY



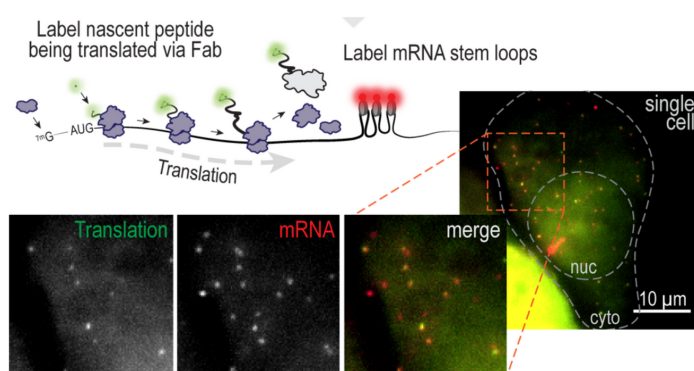
by: Divya Kolakada, PhD candidate, and Laura Baquero, Professional Research Assistant at CU Anschutz

Seeing is Believing: New Methodologies to Study Single-mRNA Translation in Live Cells

by: Charlotte Cialek, PhD candidate at Colorado State University

Imagine a way to peer into cells, see a single, translating mRNA, watch a specific factor bind to the mRNA independent of complicated recruitment or cofactors, and monitor its effect on translation for seconds to hours.

The Stasevich lab's new methodology, Tethering 'n' Translation (TnT), provides a new perspective on studying translation. It is an adaptation to Nascent Chain Tracking (*figure below*), a microscopy method that labels nascent translation and mRNA to visualize single, translating mRNA in live cells. TnT adds a tetherable element to the mRNA that specifically recruits protein fused to a tethering peptide.



Nascent Chain Tracking is an assay that uses a microscope to visualize single mRNA and their translation signals in live human cells. Data from C. Cialek, unpublished.

Often, studying a regulatory mechanism in bulk leaves results difficult to interpret, especially for mechanisms involving mRNA-to-mRNA heterogeneity, localization, or rapid, seconds-to-minutes scale dynamics. New imaging-based technology from Tim Stasevich's lab can bypass several of these problems.

The Stasevich lab demonstrated the utility of the TnT system with microRNA pathway protein Argonaute to visualize when ribosome initiation became blocked.

However, since the short tethering peptide module could be fused to virtually any protein, TnT allows for studying different regulatory pathways or different factors involved in a pathway.

A major downside to this technology is its artificial nature. Tethering a factor may not truly reflect its natural repression mechanism, and the reporter mRNA is bulky and contains many unnatural tetherable elements.

Last summer, the Stasevich lab published another new technology extending Nascent Chain Tracking to study virus translation at single mRNA. This technology does so by adding an Internal Ribosome Entry Site (IRES) between two genes to compare cap- and IRES-initiated translation on the same reporter mRNA. Since this technique can be performed in live cells, it can show ribosome recruitment to an IRES with high resolution. Applying this assay showed IRES-mediated translation upregulation at single mRNA after induced cell stress with minutes-timescale resolution.

This technology could be used to better study different IRESs from different viruses, such as the SARS-CoV-2 IRES.

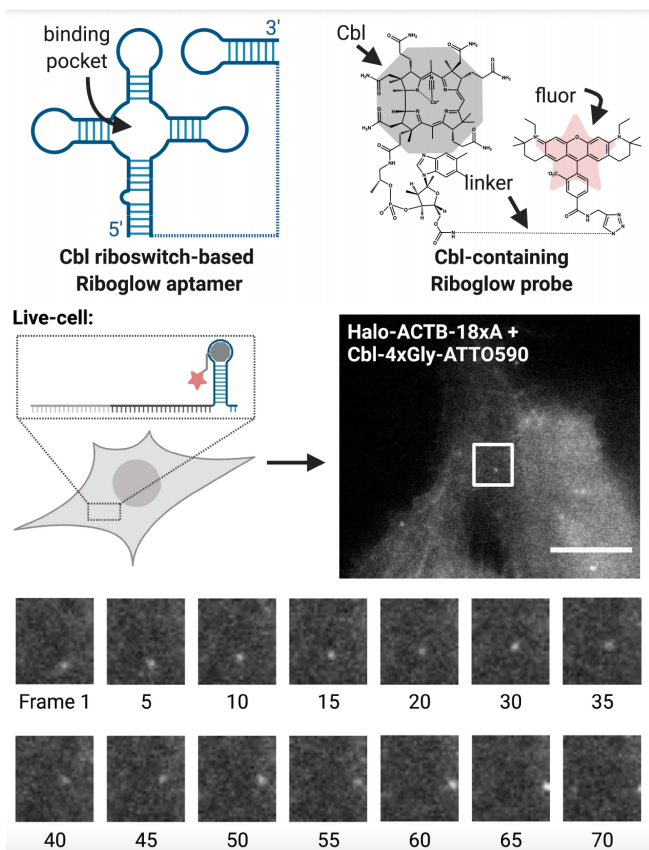
RNA's Glow Up

by: Giulia Corbet, PhD candidate at CU Boulder

RNA imaging techniques have made significant advances in recent years with the development of the MS2 and PP7 technologies, among others. However, many of these techniques have limitations, including the large size of the tag and the often accompanying protein binding partner, which can be significantly larger than the RNA itself. Moreover, attaching a large tag to an RNA can influence its localization, translation, and stability, among other things.

One technology aiming to address some of these limitations is Riboglow, a riboswitch-based imaging system developed in the labs of Amy Palmer and Robert Batey at CU Boulder. Riboglow allows for imaging of individual RNAs by tagging the RNA with a cobalamin-based riboswitch, which can bind to any of a number of cobalamin probes with a fluorescent tag (*pictured on the next page*).

The small size of the cobalamin riboswitch compared to other RNA-imaging systems may enable the visualization of smaller RNAs, such as non-coding



Schematic of the Riboglow riboswitch and cobalamin (Cbl)-linked fluorescent probe (above). Representative image of live-cell imaging of Riboglow-tagged RNA (below).

RNAs, without increasing the size of the RNA by many fold. Additionally, Riboglow does not require an RNA-binding protein partner to visualize the RNA, which avoids increasing the local protein concentration around the RNA.

The collaboration between the Palmer and Batey labs has been critical to the development of this technology, with the Batey lab providing expertise on riboswitches and the Palmer lab providing imaging expertise.

Biochemistry graduate students Erin Richards and Shelby Lennon are working collaboratively to optimize the Riboglow system. In the Batey lab, Shelby works on biochemical characterization and optimization of the riboswitch itself, while in the Palmer lab, Erin works on the imaging and in-cell characterization of

Riboglow.

Erin and Shelby (*pictured right*) are both third-year graduate students and in addition to being collaborators, they are good friends. On how their friendship has affected the

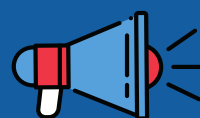
collaboration, Shelby said "I think it's enhanced the collaborative nature because beforehand, it wasn't as close...it can be intimidating to be surrounded by people that are all more experienced than you

and so that Erin and I came in at the same time and were [in] the same class is cool... we've got each other's backs." Erin added "It's very sweet because we each have our own sections of the project...but there are certain experiments that we like to do together."



Erin Richards and Shelby Lennon. Personal photo collection.

The first publication on Riboglow can be found in Nature Chemical Biology, July 2018.



CALL FOR VOLUNTEERS!

Join Our Newsletter Editorial Team!

Contribute as a writer, illustrator, or other scientific communicator.

If interested, please email ColoradoRNAclub@gmail.com or Divya.Kolakada@cuanschutz.edu



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

Organizing committee: Ankita Arora, Divya Kolakada, Giovana Breda Veronezi, Laura Baquero (CU Anschutz); Charlotte Cialek (CSU Fort Collins); Giulia Corbet (CU Boulder).

Supervision: Sujatha Jagannathan (CU Anschutz). Editor: Divya Kolakada. Design: Giovana Breda Veronezi.