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COLORADO RNA CLUB LATEST

What's new from our RNA community

LETTER FROM THE EDITOR

From the discovery of the bacterial RNA polymerase and the subsequent discovery of the three eukaryotic RNA polymerases over 50 years ago to today, our understanding of RNA transcription and processing has advanced by leaps and bounds. However, many mysteries still remain. In this issue of Colorado RNA Club's Latest, we showcase a few labs that are attempting to unravel some of these mysteries.

We feature **Tom Santangelo**, Ph.D., whose lab specializes in the study of archaeal transcriptomics, DNA replication/repair, and metabolism; **Srinivas Ramachandran**, Ph.D., whose lab, among other things, studies how nucleosome structure and dynamics affects DNA replication and RNA transcription; and **Katy Walsh**, a 3rd year Ph.D. student from the Goodrich-Kugel Lab, who studies the mechanism behind transcription termination defects caused by cellular stress. We also profile **David Bentley**, Ph.D., whose research led to the pivotal discovery that mRNA transcription and maturation are both coordinated by the C-Terminal Domain of RNA Polymerase II.

Last but not least, this will be Giulia Corbet's last issue with us. Giulia has been a regular contributor to the newsletter since its inception and has written some great articles for us in that time. We have loved having Giulia on the team and wish her the very best as she gears up to submit her thesis and graduate. We also welcome any new interest in contributing to the newsletter. If you are interested, please email divya.kolakada@cuanschutz.edu or ColoradoRNAClub@gmail.com.

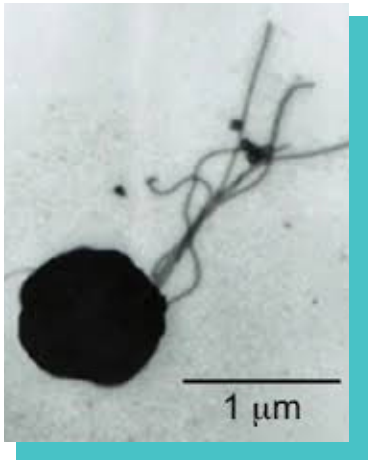
As always, we hope you enjoy reading this issue.

Divya Kolakada
Editor, Colorado RNA Club Latest

Charting the Uncharted

by: Pablo Maldonado, PhD student at CSU Fort Collins

As scientists, we are challenged to identify a niche to explore and a question that hasn't been asked in hopes of significantly contributing to the scientific community. However, creating a niche in a largely unexplored domain of biology might be most challenging. Tom Santangelo, Ph.D., did just this and jumped into the realm of archaea in 2004 with expertise in bacterial and phage transcription. "The field of transcription at the time was rapidly evolving" he says, "the very first structures of RNA polymerase were coming out, and people were stepping on each other's toes". Rather than getting caught in this hubbub for his postdoc at Ohio State University, Santangelo took interest in the unknowns of archaeal transcription in methanogens, however, genetic experimentation was difficult. By way of savvy networking, he undertook a new model — a genetically accessible archaeal hyperthermophile, *Thermococcus kodakarensis* (TKo).

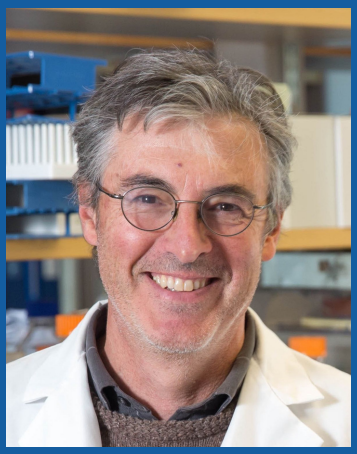


The archaeal hyperthermophile *T. kodakarensis*. Image by F. Fukuri, Kyoto University

At Colorado State University (CSU), the Santangelo lab houses a beehive of graduate and undergraduate students who maintain a clear eyed and passionate approach to understanding *T. kodakarensis*. The lab is split into three branches, each of which focus on either archaeal metabolism, DNA replication/repair, or transcriptomics with graduate students at the

Bent(ley) on mRNA Transcription and Processing

by: Divya Kolakada, PhD candidate at CU Anschutz



David Bentley, Ph.D. Personal photo collection.

David Bentley, Ph.D., strides through the 10th floor laboratories of the Biochemistry and Molecular Genetics Department at the Anschutz Medical Campus, with a scientific swagger that only he can muster. While "scientific swagger" is a peculiar turn of phrase, it is difficult to find a more suitable one. Bentley has a compelling way of speaking about science, he draws you in; he seemingly has a mind palace of papers he can pull from at any given time; his discoveries have led to a paradigm shift in transcriptional and mRNA processing research.

"I love the thrill of discovery," Bentley says, "the moments when you feel as if you have found something out about the way mother nature works that nobody else knows right then."

Research in the Bentley lab centers around mRNA synthesis and maturation: they are most known for discovering that the C-Terminal Domain of RNA Polymerase II coordinates both mRNA transcription and maturation. "That was a moment where [you realize] you've just found out something pretty important. That's a good feeling," he reminisces, "but...you can't just live for moments like that... You've got to get satisfaction from the things that happen everyday too."

Bentley believes that the next big challenge of the transcriptional/ mRNA processing field is to understand how the processes are controlled by physiological stimuli like hormones, stress, or developmental cues. "It's a complex problem and there's a lot to deconvolute. And who knows what methods and approaches might become useful there, maybe spatial transcriptomics, for example," he contemplated, " the knowledge of how signaling processes work will illuminate very much and guide, I predict, the design of future RNA therapeutics."

On aspiring to be a PI, he professes, "you do it if you don't feel as if you have a choice because you don't feel as if there's anything else that's going to give [you] the reward that [you] want from [your] life". His advice to trainees who want to pursue that path: "do it if this is what you really enjoy ... be very honest with yourself about that." His advice for new PIs: " You've got to be able to distinguish what is a really important finding and what is a finding that is actually not worth me directing a lot of my precious time and effort into ... Really good scientists realize when a project is not going somewhere and they know when to pull the plug. And my experience is that really great scientists know that early and they pull the plug early and don't get wedded to a project."

the forefront. With respect to transcription, Santangelo expressed that classic "grind and find" methods such as protein purification and in vitro transcription are still just as important for studying transcription today as they were in 2004. Fortunately for archaeal enthusiasts like Santangelo, the community settled on several model species at the turn of the sequencing boom which allowed a deep dive into their genetic underlyings. Today, the lab utilizes their newly developed sequencing technique known as ac^{4}C-seq to decipher the implications of RNA modifications in hyperthermophiles. Ac^{4}C-seq integrates biochemistry and Next Gen sequencing, forming reduced nucleobases that induce detectable non-cognate deoxynucleotide triphosphates upon reverse transcription. There's much to be understood about harboring regions in TKo. Essentially, the event of how RNA maintains its integrity at temperatures of upwards of 95°C, and he notes that RNA are critical for archaeal stability and function at various

temperatures. The lab recently published on RNA acetylation and its potential for stabilizing regions of ribosomal RNA which hold metals when not modified. "Negatively charged RNA is difficult to keep in a globular fold and are stabilized by positively charged metals" he says, "at high temperatures these stabilizing forces can be lost" noting that acetylation was critical at stabilizing metal losing a stabilizing metal at higher temperatures is compensated via acetylation. He also mentions that an entirely new modification was discovered by the lab, "by dumb luck but luck is good in science...I can't say much other than it's in the ribosome and is important for survivability".

It takes significant motivation to keep churning new areas in biology. "I think you have to be a little bit kooky to study something for so long and never get to the end, '' Santangelo says. He attributes his success in the field to "insatiable curiosity" as well as his student's hard work. When asked about his motivation as a mentor of his many students he says, "somebody has to give people a chance to actually do science"–that those brave enough to dedicate themselves to the craft should be rewarded.

When The Nucleosome "Fights Back"

by: Jillian Ramos, PhD, Postdoctoral Scholar at CU Anschutz

Srinivas Ramachandran, Ph.D. Personal photo collection.

When I think about RNA transcription, a few terms that quickly pop into my head are RNA polymerases, transcription factors, etc. It wasn't until I sat down and spoke with Srinivas Ramachandran, Ph.D., an Assistant Professor at Anschutz Medical Campus, did I take a step back and ponder the role of nucleosomes in transcription. Continue reading to learn how the Ramachandran Lab's research led me to think about transcription in an entirely different way.

In our discussion, Ramachandran explained that many DNA-binding proteins recognize a specific sequence and bind only a few locations in the genome, but that is not true for nucleosomes. Nucleosomes need to be capable of being everywhere on a DNA and be dynamic to allow for DNA replication and RNA transcription. Ramachandran's lab studies both the structure and dynamics of nucleosomes using a combination of sequencing, biochemistry, and data science. One example of his lab using classical biochemistry to ask genomic questions includes using micrococcal nuclease digestion followed by sequencing. Micrococcal nuclease degrades exposed DNA leaving behind footprints indicative of the presence of nucleosomes. The varying footprints, which can range from 70-147nt, are indicative of a nucleosome's structure during specific states. Identifying and comparing the footprints during different states, such as gene activation and gene repression, allow for a better understanding of how these different states are maintained.

SAVE THE DATE!

Colorado RNA Club 2nd Annual Industry Session

INDUSTRY CAREER PANEL

FEATURING:

- **Lightning Talks**
- **Panel Discussion and Q&A** - Industry representatives from Myriad Genomics, Terumo Blood and Cell Technologies, Curry Rockefeller Group, and more!!
- **Zoom Breakout Rooms** - fill out the form in the QR code below about career paths you would like to learn about.

WHEN: TUESDAY, MARCH 29TH FROM 2-4 PM MST

FORMAT: Digital only

ZOOM MEETING ID: 999 3661 8858

PASSWORD: 204546

Furthermore, nucleosome dynamics should allow processes such as replication and transcription, both of which occur at high speeds and need access to naked DNA. Both processes occur efficiently in the cell, pointing to routine and rapid unwrapping and re-wrapping of nucleosomal DNA. An interesting observation in work from the Ramachandran Lab has been at regulatory sequences. Ramachandran said:

"Our current model is that transcription factors cooperate to evict the nucleosome, but the nucleosome 'fights back'", because the goal of a nucleosome is to prevent DNA from being exposed, as this exposure can lead to casualties such as DNA damage and aberrant transcription."

Another area of research in Ramachandran's lab is to identify chromatin dynamics in humans using circulating cell free DNA (cfDNA) in blood so that these dynamic profiles could in the future be used as early indicators of disease. In short, when cells die, the DNA is exposed to nucleases and the DNA fragments end up in the blood. His pilot grant from the University of Colorado Cancer Center aims to identify how age affects chromatin structure as reflected in the cfDNA found in the blood. In the future, the goal is to also use this chromatin information to determine how individuals respond to therapies.

When we discussed the future of the field, Ramachandran mentioned that the next frontier is to comprehensively map nucleosome dynamics in single cells. While single-cell sequencing is possible now, it is still challenging for high-resolution mapping of chromatin as there are only two DNA copies in the genome and while high-resolution imaging has been revolutionary in understanding genome structure, it is still not easy to map all parts of the genome. Overall, Ramachandran said that this work and his field are important because answering these questions "is essential to understanding cellular identities, which are key to understanding how we are made".

HIGHLIGHTED PUBLICATIONS

Evolutionary divergence of Firre localization and expression

Rinn lab - CU Boulder, Mar/2022

The evolution of DUX4 gene regulation and its implication for facioscapulohumeral muscular dystrophy

Jagannathan Lab - CU Anschutz, Feb/2022

Small RNA pathways in the nematode Ascaris in the absence of piRNAs

Davis lab - CU Anschutz, Feb/2022

Merging Established Mechanisms with New Insights: Condensates, Hubs, and the Regulation of RNA Polymerase II Transcription

Taatjes lab - CU Boulder, Jan/2022

Analysis of subcellular transcriptomes by RNA proximity labeling with Halo-seq

Taliaferro lab - CU Anschutz, Feb/2022

A plant-infecting subviral RNA associated with poleroviruses produces a subgenomic RNA which resists exonuclease XRN1 in vitro Wilusz lab - CSU Fort Collins, Jan/2022

Are stress granules the RNA analogs of misfolded protein aggregates?

Parker lab - CU Boulder, Jan/2022

Single-Molecule Imaging of mRNA Interactions with Stress Granules

Stasevich lab - CSU Fort Collins, Jan/2022

Transcription Termination Gets Stressful

by: Giulia Corbet, PhD candidate at CU Boulder

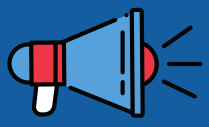
Katy Walsh. Personal photo collection.

Cellular stress in the form of heat shock, osmotic stress, or viral infection induces a variety of cellular responses including, but not limited to, altered metabolism and gene expression. A lesser understood consequence of cellular stress is the phenomenon known as transcription-termination defects, which are the focus of 3rd year Biochemistry Ph.D. student Katy Walsh's research.

When cells undergo stress, RNA Polymerase II (Pol II) does not terminate transcription at the end of a gene as it normally does; rather, it continues transcription downstream of the gene. This transcription can run on for thousands of bases and often goes into the next gene. It is not known how or why these transcription-termination defects occur. In the Goodrich-Kugel lab at CU Boulder, Walsh is focused on understanding the mechanisms behind transcription-termination defects. "One of our initial hypotheses is that the C-terminal domain of Pol II is being differentially phosphorylated under stress... the C-terminal domain in general is important for controlling various stages of transcription and interfacing with other co-transcription factors [and] termination factors," Walsh explained.

The primary approach Walsh is using to test this hypothesis is phospho-specific chromatin immunoprecipitation sequencing (ChIP-Seq). ChIP-Seq allows Walsh to look at where in the gene Pol II gets phosphorylated, if at all, and how that changes during stress. While heat shock is the model system Walsh uses at the moment, it will be interesting to understand whether the mechanisms causing transcription-termination defects of Pol II areconserved across different stresses.

Another interesting question is what is the fate of the run-on transcripts that are being produced? "We're not sure what the implications of having really long chimeric RNA products would have. We don't know if potentially some of the normal processing is happening...It's a big unknown question of why this is happening [and] if it's beneficial for cells," Walsh said. Using 4-thiouridine (4-SU) to label and sequence newly transcribed RNAs, Walsh will be able to identify what RNA species are produced during stress and determine whether RNA cleavage is occuring. These experiments will further our understanding of whether transcription run-on and its RNA products are beneficial to the stress response or whether they are a consequence of stress.



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Organizing committee: Ankita Arora, Divya Kolakada, Giovana Breda Veronezi, Jillian Ramos, Nicole Moss (CU Anschutz); Pablo Maldonado (CSU Fort Collins); Giulia Corbet (CU Boulder).
Supervision: Sujatha Jagannathan (CU Anschutz). Editor: Divya Kolakada. Design: Giovana Breda Veronezi.