March 2021 Volume No. 3

COLORADO RNA CLUB LATEST

What's new from our RNA community

In this issue: What is the RNA World Hypothesis? | Alumni Spotlight | Grandma DAP's Instructions for Life | Is it Time to Refine the RNA World Hypothesis? | Opinions from the Community | Announcements

What is the RNA World Hypothesis?

by: Giulia Corbet, PhD candidate at CU Boulder

As far as we know, all forms of life on earth use DNA as their genetic material. However, that likely has not always been the case. The RNA World Hypothesis posits that RNA existed prior to DNA and proteins, and may have served as the first genetic material. The hypothesis proposes that random chemistry in prebiotic times led to the synthesis of nucleotides, which then polymerized into ribonucleic acids.

Several properties of RNA make it a plausible candidate for supporting early forms of life. First, in addition to storing genetic information, RNA is capable of self-replication. DNA, on the other hand, requires protein for both replication and for RNA transcription. Additionally, RNA can function as a catalyst, including for the process of protein synthesis. Thus, it may be that the existence of RNA preceded the development of DNA and protein.

However, the RNA World Hypothesis has limitations, including that RNA may have been too "sticky" to have served as the genetic molecule for life on its own. The tendency of nucleic acids to self-hybridize begs the question of how these molecules were separated to facilitate replication. Furthermore, it has been argued that RNA is too complex a molecule; one that requires too many specific chemical reactions to have arisen in the prebiotic world. Some scientists postulate that an alternative self-replicating polymer must have existed first, which then may have facilitated the synthesis of the first RNAs.

Recent work spearheaded by Eddy Jiménez from the Krishnamurthy Lab at Scripps Research Institute suggests that RNA and DNA may have coevolved in primordial times. The lab shows that diamidophosphate (DAP), a molecule that may have been present in the prebiotic world, facilitates the synthesis of both ribonucleosides and deoxyribonucleosides under similar conditions. Previous work from the same group also found that RNA-DNA chimeras hybridize less strongly than RNA-only duplexes and DNA-only duplexes, therefore potentially facilitating their separation for replication. Thus, the so-called "chicken or the egg" dilemma of RNA and DNA may not be the right question after all.

Alumni Spotlight: Rebecca Turk MacLeod, Ph.D.

by: Divya Kolakada, PhD candidate at CU Anschutz

In this issue's alumni spotlight, we spoke with Rebecca Turk MacLeod. MacLeod is a former student of the Yarus Lab at CU Boulder, who's PhD work had implications for the RNA World Hypothesis. She currently works as Scientist 2 at Illumina. This is her journey.

Dr. Rebecca Turk MacLeod, Personal Photo Collection

Rebecca Turk MacLeod is a free spirit who has never wanted to be constrained by location in order to follow her passion. As a lover of both astronomy and biology, she moved from New Hampshire to Colorado to pursue a seemingly unusual field for her Ph.D., namely, Astrobiology.

"I think a lot of people, when they first hear about [Astrobiology] … [think] that's crazy: you're just looking for little green men," MacLeod said wryly,

Dr. Rebecca Turk MacLeod during her graduation from CU Boulder, Personal Photo Collection

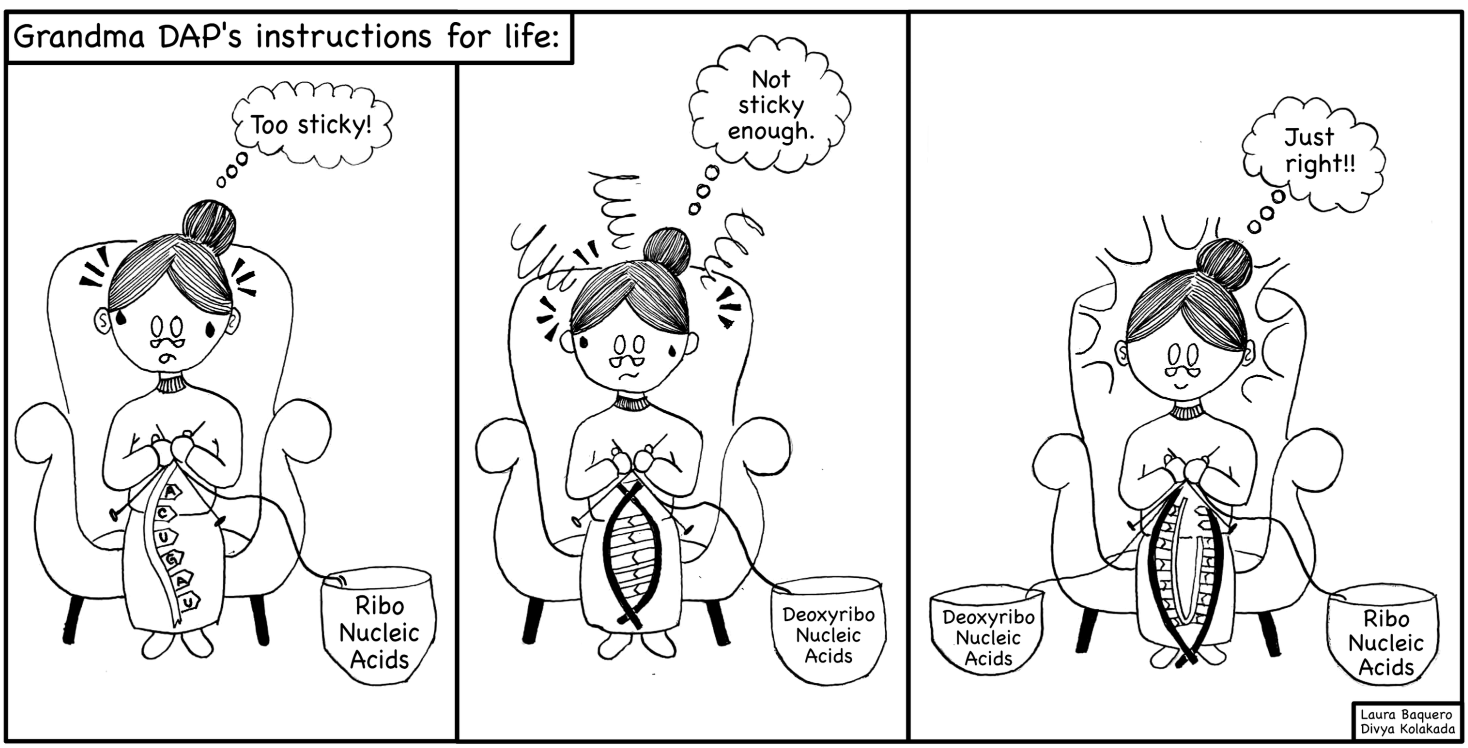
"[But] … it's really, on a fundamental level, looking at what is life, what makes life, … how plausible is it that we could actually find life out there, what would we look for, [and] how do we find it. It is I think, still one of the most profound scientific questions: are we alone in the universe?"

Her passion led MacLeod to join the lab of Michael Yarus. Here, what was initially a side project took over MacLeod's dissertation. She discovered a five nucleotide ribozyme that could perform the function of an aminoacyl transferase. "It was kind of a big deal," she stated, "but the point I want to make is that it wasn't my initial project … It's never going to go the way you think … and that's ok."

As MacLeod was preparing to graduate from CU Boulder, she became interested in microfluidics through interactions with Dr. Andrew Griffiths. Griffiths was a professor at ESPCI Paris who had studied RNA evolution in microdroplets. Since microdroplets could model protocells, using them to study prebiotic interactions made complete sense to MacLeod. The pair applied for four fellowships. In their first attempt they weren't offered any and MacLeod instead began a postdoctoral fellowship at Harvard University under Dr. Irene Chen. In their second attempt the next year, however, they were offered three.

"There is nothing amazing that happened during that time between the first and second year," MacLeod reflected on her time at Harvard, "[so], what happens, it's not necessarily a reflection on you."

Not one to be constrained by location, she picked the postdoctoral fellowship offered by the Human Frontier Science Program and moved her family to



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

Paris. She spent two years in the Griffiths Lab before moving to University of Glasgow, Scotland to work with Dr. Leroy Cronin. Here, her project still involved microfluidics, but it was more on the engineering and software development side, and she was loving every minute of it. Unfortunately, due to visa issues, MacLeod and her family had to move back to the US.

MacLeod had long cherished dreams of becoming a professor. Although she applied for several faculty positions, it did not end up working out. She now works as Scientist 2 at Illumina in materials and surface science. Although rueful that she did not get to be a professor, she loves her job at Illumina. She particularly appreciates the financial stability, security, and flexibility her job offers while allowing her to do the thing she loves – science.

Her advice for those who want to transition to industry: Networking.

“It basically means don’t burn any bridges if you can help it, keep everybody in your contacts, [and] don’t be afraid to contact people out of the blue,” she explained, before adding some personal advice, “Please don’t feel like a failure ... You can have a good, fulfilling, happy, and worthwhile career even if it’s not the thing you originally envisioned yourself doing.”

Is it Time to Refine the RNA World Hypothesis?

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

4 billion years ago, the first molecular precursors to life emerged in Earth’s primordial soup of chemicals. However, the identity of these molecules remains a subject of heated debate, although the consensus is that they had to perform two basic functions - storing information and catalyzing chemical reactions. A promising candidate was RNA - a molecule shown to have both replicative and catalytic function - that would then pave the way for DNA and proteins to take over later.

A recent study argues that RNA strands alone are too sticky to perform the replicative function and provide evidence that a chimeric molecule with part DNA and part RNA solves the problem. “Often in the field of origin-of-life, a breakthrough in understanding

OPINIONS FROM THE COMMUNITY

In light of recent work published by the Scripps Research Institute (see our “What is the RNA World Hypothesis?” article for details), we reached out to members of our community to get their perspectives on this research topic. Here are their responses.

"A Mixed-Backbone Nucleic Acid World?
While the RNA World is an engaging hypothesis, we should anticipate that it was preceded by an initial form of nucleic acid polymers with backbones of mixed sugars, a Mixed-Backbone Nucleic Acid World. This is suggested by observations that nucleic polymers with mixtures of ribose and deoxyribose backbones can have catalytic properties similar to RNA polymers, that the formose reaction (the most likely form of prebiotic sugar synthesis) produces mixtures of carbohydrates, and that the prebiotic coupling of sugars to phosphates, purines, or pyrimidines will be similar for different carbohydrates. This suggests a model that replicating molecules first emerged with mixtures of carbohydrate backbones, and that selection favored the ultimate utilization of ribose as the prevailing sugar, perhaps either because of superior catalytic or replicative properties, or because of the propensity of ribose to enter primitive lipid proto-cells."

Dr. Roy Parker - CU Boulder

"This ms will interest nucleic acid chemists – a summary in chemical language is that a novel activator DAP (diamidophosphate, and in the required presence of 2-aminoimidazole) will not only activate the 5’ P of a deoxynucleoside, but the activated deoxy goes ahead, without further additions, to form short DNAs, up to about tetramers. And as a further attractive wrinkle, activation seems to be stimulated by base-pairing partners. All this is encouraging to early-DNA advocates interested in the origin of life. Cautions might be that the conditions are a bit demanding (many components - solutions don’t work, semi-dry pastes are required). But it is early days yet, and if you are interested in this area, it is very clear and convincing: have a look."

Dr. Michael Yarus - CU Boulder

(cont. p4)

OPINIONS FROM THE COMMUNITY

"Was the RNA World a DNA-RNA World?
According to the RNA World hypothesis, the first pre-biotic self-replicating system consisted of RNA. Its plausibility derives from RNA serving as both genotype and phenotype, both information and function (Carl Woese). Proteins were incorporated later and, finally, DNA.

I've been suspicious of origins-of-life research, because a laboratory experiment cannot answer a historical question. It can only say what is possible, not what actually happened 3.8 billion years ago. But Leslie Orgel, one of my mentors, explained that if RNA World research reveals fundamental principles of nucleic acid chemistry and self-assembly, then it's a worthwhile endeavor even if the big question is unanswerable.

Krishnamurthy et al. (Angew. Chem. Int. Ed., 2020) have now shown that deoxynucleosides can be phosphorylated and oligomerized under plausible prebiotic conditions, leading to speculation that a mixed DNA-RNA copolymer could have been a prebiotic genetic material.

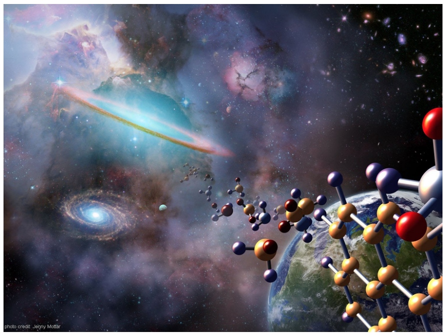
Their work is chemically rigorous and interesting, but it doesn't necessarily affect the RNA World hypothesis. As explained by Jerry Joyce and Leslie Orgel (The RNA World, 1999), RNA is probably too complex to have been the first prebiotic nucleic acid, so "the presumed RNA World should be viewed as a milestone, a plateau in the early history of life on earth." If chimeric DNA-RNA copolymers came earlier, perhaps their inability to form good ribozymes set them up for failure, and RNA took over.

Another reason to give a circumspect eye to this new work is that the research uses pure deoxynucleosides purchased from Sigma-Aldrich, thereby short-circuiting issues of prebiotic synthesis of the monomers and of chiral selection (polymerization of D-enantiomers can be poisoned by the presence of L-enantiomers). Sigma-Aldrich was not delivering in prebiotic times.

Thus, many deep challenges remain in the quest to achieve self-replicating nucleic acids in the test tube."

Dr. Tom Cech - CU Boulder

biological processes leads to refining the RNA World Hypothesis", said Quentin Vicens, PhD Research Assistant Professor at the University of Colorado-Anschutz Medical Campus. "It is highly unlikely that a single polymer (RNA) could carry out all of the necessary processes required for life to sustain. Nor that it would have needed to, because there were plenty of other molecules around: modified bases, alternative sugars, amino acids, small peptides, other small molecules..."



NASA / Jenny Mottar

The above idea is still in agreement with the model that RNA might have played an important role of acting as the main driver but with much needed assistance from these other small molecules. What is needed is the evidence to prove the hypothesis, which is next to impossible given we can't time travel. We can't know for sure what the early environments were really like and we'll never know exactly what really happened, but that doesn't mean the questions aren't worth asking. "The RNA world hypothesis helps us think about what is necessary to sustain life. So that for example we would be able to find it outside Earth - this is where astrobiology plays a pivotal role", said Vicens. "It is still possible the fossil of an RNA organism has not been discovered yet - what Peter Moore and Mike Yarus humorously called '**a Ribosaur**'!"

"The most compelling evidence is the ability of RNA to evolve functions/binding capabilities under selection pressure - a property shown in the laboratory using SELEX (In Vitro selection) experiments under prebiotic conditions, which mimic the Darwinian evolution.", said Vicens.

What is needed as the next step is to think about how to go from prebiotic chemistry to very simple RNA-based cellular structures. A primitive cell often called a 'protocell' has two parts: a fat-rich boundary that self-assembles into a spherical vesicle and encapsulates genetic molecules in the internal, aqueous compartment. The fact that even with our advanced technologies we haven't yet proven the existence of a protocell that can self-replicate, grow and divide tells us that we're still far from the molecular answers we seek.

"Now that we understand better how a primordial chemistry could have made the first RNAs and DNAs, we can start using it on mixes of ribonucleoside and deoxynucleoside building blocks to see what chimeric molecules are formed—and whether they can self-replicate and evolve," study senior author Ramanarayanan Krishnamurthy, associate professor at Scripps Research told The Scripps Institute news release.

One of the key questions that is often ignored and needs research attention is, What was the energy source for the RNA World? And, how was it possible to link a stable energy supply to a metabolic synthesis of RNA?

With advancements in biology, from the seminal Miller-Urey experiment to the discovery of RNA aptamers, riboswitches and ribozymes - the RNA world hypothesis has evolved through time. This discovery of a chimeric genetic material just opens a new avenue to explore for RNA scientists interested in understanding the transition from a pre-life chemical soup to the living protocell.

ANNOUNCEMENTS

COLORADO'S FIRST HANNA GRAY FELLOW

by: Danielle Bilodeau, PhD candidate at CU Anschutz

It is with great pride that the Colorado RNA Club wishes to congratulate **CU Anschutz's Dr. Steve Bonilla** on receiving a **Hanna Gray Fellowship** from the Howard Hughes Medical Institute.

Dr. Steve L. Bonilla, HHMI.org

Dr. Bonilla is **one of only 21 scientists from across the country** to receive this prestigious award, which will provide him with four years of post-doc funding and a further four years of funding to set up his own lab. Of the now 43 Hanna Gray Fellows selected in the past 4 years, Dr. Bonilla is the **first to represent a research institution in Colorado**.

RNA CLUB INDUSTRY SESSION

by: Giulia Corbet, PhD candidate at CU Boulder

Don't miss out a great opportunity to get exposure to **industry science** and learn about **career paths outside of academia**!

- **Short talks from industry** - featuring speakers from Moderna, Horizon Discovery, and Eclipse Bioinnovations, followed by **Q&A session.**
- **Breakout session with Alumni** - fill out the form in the QR code below about which career paths you would like to learn about.

WHEN: TUESDAY, MARCH 30TH FROM 2-4:30 PM

ZOOM MEETING ID: 977 2423 4174

PASSWORD: rnaclub

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, Danielle Bilodeau, Divya Kolakada, Giovana Breda Veronezi, Laura Baquero (CU Anschutz); Charlotte Cialek (CSU Fort Collins); Giulia Corbet, Soraya Shehata (CU Boulder).
Supervision: Sujatha Jagannathan (CU Anschutz). Editor: Danielle Bilodeau. Design: Giovana Breda Veronezi.