

Aliens among us: Bacteriophages that use alternative genetic codes

Miller Fellow Focus: Adair Borges

I study bacteriophages (phages), which are the viruses that infect bacteria. Viruses in general are really interesting, because they are very diverse, incredibly abundant, and rapidly evolving. Phages exist in an ancient evolutionary arms-race with their bacterial hosts, where each creature tries to destroy the other. Over eons of evolutionary time, both phages and bacteria have pushed each other to great heights of biological innovation.

I got my start researching phages in the laboratory, and in my thesis work I studied how phages survived being attacked by the bacterial CRISPR-Cas immune system. We learned that phages had evolved many different strategies to shut down this powerful immune system: some cooperated as a team to take over the cell¹, while others used molecules and strategies that enabled them to hunt alone². Throughout my PhD work, I never ceased to be thrilled by the creativity and invention that phages could exhibit.

However, the vast diversity of phages in the world cannot be studied in the laboratory. Instead, scientists use metagenomics to discover new and exciting phages from many different environments. Metagenomics relies on sequencing of DNA collected from natural microbial communities - a complex mix of bacteria, bacteriophages, archaea, fungi, protists, and other viruses. Then, computational tools can be used to patch



these DNA sequences back together into computerized representations of individual organisms.

Jill Banfield's lab at UC Berkeley is one of the world leaders in using metagenomics to expand the bacteriophage universe. During my PhD studies I was amazed to read their reports of the metagenomic discovery of giant phages that used genetic codes different from their host bacteria^{3,4}. It was this exciting finding that would ultimately inspire my own project as a Miller Fellow, hosted by Jill Banfield's lab.

The genetic code is a highly conserved feature of life on Earth. All known organisms use a genetic code of triplet combinations of DNA letters, called

INSIDE THIS EDITION

Miller Fellow Focus	1-3
2022 Professorship Awards	4
In the News	3, 5
Recent Publications	5
Gifts to the Miller Institute	6
First Annual Fall Retreat & Annual Fall Dinner 2021	7
Miller Picnic & Hike 2021	8

The Miller Institute is "dedicated to the encouragement of creative thought and the conduct of research and investigation in the field of pure science and investigation in the field of applied science in so far as such research and investigation are deemed by the Advisory Board to offer a promising approach to fundamental problems."

"Frankly, I think choosing the Miller over other fellowships was one of the best decisions I made. The Miller Fellowship 1) provided me the academic freedom to explore topics that interested me, 2) introduced me to a community of brilliant people and 3) provided unintrusive administrative support. I will certainly look back fondly on these years."

- Alex Turner

Miller Fellow 2017-2020
Assistant Professor,
University of Washington,
Department of Atmospheric Sciences



CONTINUED ON PAGE 2 >



codons, to instruct the protein synthesis machinery of the cell to make proteins. It makes no sense for phages, which canonically rely on the bacterial host for protein synthesis machinery, to use a genetic code that the bacterial machinery cannot interpret.

When I started as a Miller Fellow hosted in Jill's lab, I had limited prior knowledge of metagenomics and bioinformatics. However, under Jill's tutelage, and with support from lab members (especially graduate students Clare Lou and Alex Jaffe), I became comfortable studying phages in this new way. I also found support for my work on alternative genetic codes within the Miller community, in collaboration with Miller Professor Yun Song and fellow Miller Fellow Grayson Chadwick.

In starting this project, there were many open questions: How many phages use alternative genetic codes? How do such codes evolve? And most importantly, why would a phage ever use an alternative genetic code in the first place?

In a recent preprint⁵, we try our best to answer these questions. Here, we identify numerous examples of stop codon recoding in the human and animal gut microbiome. In this scenario, either the TAG or TGA stop codon had been reassigned to encode for an amino acid (Figure 1). This type of recoding is pretty extreme, because it means that the bacterial protein production machinery should be instructed to stop many times throughout the length of a recoded protein. When we used standard computational methods to predict the proteins encoded by each phage, these phages were predicted to have very low coding density because their proteins were constantly being fragmented by these recoded stop codons.

total, I found that between 2-6% of the bacteriophage genomes in human and animal gut microbiomes use recoded stop codons.

Next, we wanted to figure out how stop codon recoding evolves. To do this, we tracked down relatives of alternatively coded phages that used the standard genetic code. We were very surprised at what we found: some groups of alternatively coded phages had super close relatives that used standard code! In fact, some families of phage seemed to be changing their genetic code all the time. So not only did a decent number of phages use alternative genetic codes, but these new codes were popping up in phage lineages on very rapid timescales.

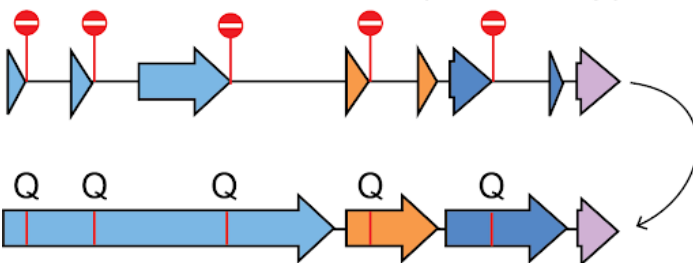
To me, this rapid evolution had to mean that stop codon recoding was good for something. And indeed, when I carefully analyzed phage genomes with recoded stop codons, I got some hints as to what this benefit might be. These phages assiduously avoided using recoded stop codons in some parts of their genomes, while other regions of their DNA were chock full of recoded stop codons. In mapping these patterns of code use across many phages, it became clear that phages were putting recoded stop codons in all their "late" expressed genes and avoiding stop codons in their "early" genes.

Phages care a lot about gene regulation. Within a short period of time (around 60 minutes for many phages) a phage will take over the cell, copy its DNA many times, assemble new phage particles, and ultimately explode the cell to release its progeny. As you can imagine the order of operations is very important here: if a phage explodes the cell before it successfully assembles its progeny phages, that phage lineage goes extinct. A statistical analysis of stop codon use across phage genes showed that the toxic cell-exploding genes strongly preferred to use recoded stop codons. We think that by filling these time-sensitive genes with molecular stop signs, the phage prevents any chance that they could be produced out of turn.

A final question is the mechanism by which the phage changes the code of the cell. In about half of alternatively coded genomes we found a transfer RNA gene which would be predicted to decode stop codons. In very rare cases, we were able to find machinery that would load those transfer RNAs with amino acids. We predict that the phage produces these "code change" gene products right before it wants to enter the final phase of its life cycle. We suspect that alternatively coded phage genomes are a treasure trove of unusual and new "code change" molecules waiting to be discovered.

To sum it all up, stop codon recoding is widespread in uncultured phages of the gut microbiome, where we believe it functions as an extreme form of codon-based gene

Standard code translation (TAG = Stop)



Alternative code translation (TAG = Q)

Figure 1: The TAG codon normally signals for the protein to stop being made, but some phages use it to code for the amino acid glutamine (Q). The top panel shows alternatively coded phage proteins being fragmented in standard code, and the bottom panel shows those proteins translated with an alternative code.

While this fragmentation creates an obvious problem for phage gene prediction, it is a useful indicator of stop codon recoding. I took advantage of this, and searched thousands of phages for genomes with low coding densities. In

regulation (**Figure 2**). Ultimately this work highlights exactly why fundamental research on phages is critical to tapping into the full potential of biology: phages are nimble evolvers who can explore evolutionary paths less traveled by cellular life.

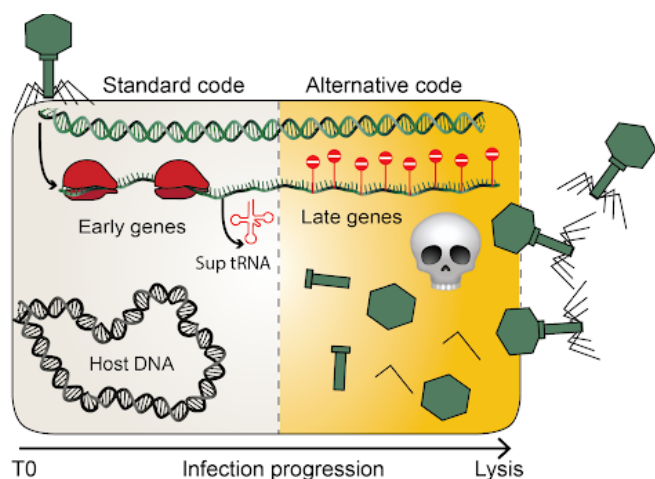


Figure 2. When an alternatively coded phage infects a bacterial cell that uses standard code, proteins are initially produced from standard code compatible “early” genes. During this phase, translation of alternatively coded “late” genes is prevented by in-frame recoded stop codons. After production of a suppressor tRNA (Sup tRNA) that can decode alternative code, the gene products initially disrupted by in-frame stop codons can be produced. This allows for expression of phage structural proteins and ultimately triggers lysis and cell death.

References

1. Borges, A. L. *et al.* Bacteriophage Cooperation Suppresses CRISPR-Cas3 and Cas9 Immunity. *Cell* **174**, 917–925.e10 (2018).
2. Mendoza, S. D. *et al.* A bacteriophage nucleus-like compartment shields DNA from CRISPR nucleases. *Nature* **577**, 244–248 (2020).
3. Devoto, A. E. *et al.* Megaphages infect *Prevotella* and variants are widespread in gut microbiomes. *Nat Microbiol* **4**, 693–700 (2019).
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5. Borges, A. L. *et al.* Stop codon recoding is widespread in diverse phage lineages and has the potential to regulate translation of late stage and lytic genes. *bioRxiv* 2021.08.26.457843 (2021) doi:10.1101/2021.08.26.457843.

Adair Borges is from Billings, Montana, and completed her undergraduate degree in Microbiology at University of Pittsburgh in Pennsylvania. She then moved to San Francisco to get her PhD studying bacteriophages in the brand new lab of Joe Bondy-Denomy at UCSF. Since 2020, Adair has been a Miller Fellow, researching the evolution of alternative genetic codes with Jill Banfield. When Adair is not studying bacteriophages, she enjoys exploring the Bay Area with her partner and hanging out with their cat Cabbage. In January 2022, Adair will start as a Scientist at Arcadia Science in Berkeley, CA.
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In the News

(see more current & past Miller Institute news: miller.berkeley.edu/news)

President Biden appointed **Saul Perlmutter** (Miller Senior Fellow 2010-2015) & **Inez Fung** (Miller Professor 2016-2017) to the President's Council of Advisors on Science and Technology (PCAST). "... Because of their extraordinary intellect, their wide range of experiences and unprecedented diversity, this PCAST will see new possibilities to create good jobs and to power American workers, and grow the economy for everyone. To change the course of human health and disease, to tackle the climate crisis with American innovation, and to lead the world in technologies and industries of the future to protect our security."

Lou Barreau (Miller Fellow 2018-2020) was awarded the **2021 Louis Armand Prize in Chemistry from the French Academy of Sciences** for "her studies of the fundamental dynamics of electrons and atoms in molecules..."

Eliezer Rabinovici (Visiting Miller Professor Fall 2002) was elected the **24th President of the CERN Council**. Professor Rabinovici's main field of research is theoretical high-energy physics and, in particular, quantum field theory and string theory. He has made major contributions to the understanding of the phase structure of gauge theories, which are the building blocks of the Standard Model, and the uncovering of the phases of gravity.

David Julius (Miller Institute's 2018 Symposium Speaker) and **Ardem Patapoutian** were awarded the **2021 Nobel Prize in Physiology or Medicine** "for their discoveries of receptors for temperature and touch."

Norman Yao (Miller Fellow 2014-2017) was honored with the **Breakthrough New Horizons in Physics Prize** "for pioneering theoretical work formulating novel phases of non-equilibrium quantum matter, including time crystals."

The **2021 Line and Michel Loève International Prize in Probability** was awarded to **Ivan Corwin** (Visiting Miller Professor Fall 2021.) "Awarded every two years since 1993, it is intended to recognize outstanding contributions by researchers in probability who are under 45 years old."

Frederick Matsen IV (Miller Fellow 2007-2010) & **Mikhail Shapiro** (Miller Fellow 2011-2013) are members of the new cohort of the **2021 HHMI Investigators** selected from more than 800 eligible applicants! Frederick Matsen is working on creating computational algorithms to analyze large sets of genetic data from an evolutionary perspective, as well as applying his mathematical wizardry to immunology. Mikhail Shapiro and his team are pioneering a method that uses ultrasound to image and track cells in living animals.

NEWS CONTINUED ON PAGE 5 >

Miller Research Competitions: Awards

On November 30, 2021, the Advisory Board of the Miller Institute met to select next year's Professorship awards. The Board is comprised of four advisors external to UCB: Luis Caffarelli (Mathematics, University of Texas, Austin), Scott Edwards (Evolutionary Biology, Harvard), Feryal Özel (Astronomy & Physics, University of Arizona) and Tim Stearns (Biology, Stanford University); and four internal Executive Committee members: Executive Director Marla Feller (Molecular & Cell Biology), Roland Bürgmann (Earth & Planetary Science), Chung-Pei Ma (Astronomy & Physics) and Yun Song (EECS/Statistics/IB). The Board is chaired by Chancellor Carol Christ.

The Miller Institute is proud to announce the awards for Professorship terms during the Academic Year 2022-2023. These outstanding scientists pursue their research, following promising leads as they develop. The Visiting Miller Professors join faculty hosts on the Berkeley campus for collaborative research interactions.



Miller Professorship Awards

Benjamin Recht
EECS

Alanna Schepartz
Chemistry

Ting Xu
MSE & Chemistry

Visiting Miller Professorship Awards

Pietro Caputo
Statistics

Host: Alistair Sinclair

Home Institution: University of Rome III

Andrew Marks
Mathematics

Host: Antonio Montalban

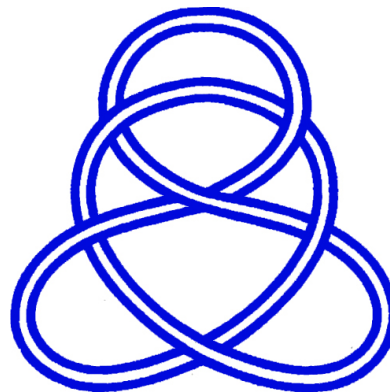
Home Institution: UCLA

Rob Phillips

Molecular and Cell Biology

Host: Hernan Garcia

Home Institution: Caltech



Joan Redwing

Materials Science and Engineering

Host: Zakaria Al Balushi

Home Institution: Pennsylvania State University

Alan Robock

Earth & Planetary Science

Host: David Romps

Home Institution: Rutgers University

Vahid Sandoghdar

Physics

Host: Naomi Ginsberg

Home Institution: Max Planck Institute
for the Science of Light



In the News

Research collaboration between ExxonMobil's team and **Jeffrey Long's** (Miller Professor 2011, 2021-2022) UC Berkeley team led to discovery of a new material that could capture more than 90 percent of CO₂ emitted from industrial sources.

Richmond Sarpong (Miller Professor 2017-2018) received the **2021 Edward Leete award** from the Division of Organic Chemistry of the American Chemical Society in recognition of his outstanding contributions to teaching and research in organic chemistry.

Alison Galvani (Miller Fellow 2002-2004) authored a study that modeled the impact of the United States vaccination program by considering two hypothetical scenarios for caseloads and deaths. The study found that COVID-19 vaccine rollout has saved 279,000 lives.

Steven Louie (Miller Professor 1986-1987, 1995) and **Peidong Yang** (Miller Professor 2009) were elected **Foreign Academicians of the Chinese Academy of Sciences**.

Rebecca Heald (Miller Professor 2009-2010) was awarded the **2021 American Society for Cell Biology (ASCB) Keith R. Porter Lecture** as an outstanding and innovative leader at the forefront of cell biology, who is actively contributing fundamental new knowledge to our understanding of cell biology.

Kathleen Collins (Miller Professor Spring 2011, Executive Committee 2014-2015) was awarded the **2022 Earl and Thresa Stadtman Distinguished Scientist Award** from the American Society for Biochemistry and Molecular Biology (ASBMB) "*for outstanding achievement in basic research. Collins has studied telomerase structure and function for almost three decades.*"

Yi Zhang (Miller Fellow 2021-2024) was awarded the **2021 Peter B. Wagner Memorial Award for Women in Atmospheric Sciences** by the Desert Research Institute (DRI) "*based on her outstanding research addressing knowledge gaps in model projections of extreme heat in tropical regions.*"

Shana Kelley (Somorjai Visiting Miller Professor Fall 2017) and **Edward Sargent** (Somorjai Visiting Miller Professor Fall 2017) joined Northwestern's Department of Chemistry and Department of Biomedical Engineering, and are affiliated with the International Institute for Nanotechnology.

Naomi Ginsberg (Miller Professor 2017-2018) was elected a fellow of the **American Physical Society** for the "*innovative development of spatiotemporally resolved imaging and spectroscopy methods, and for their use in elucidating energy transport in hierarchical and heterogeneous materials, as well as in the formation and transformation of said materials.*"

Zahid Hasan (Visiting Miller Professor 2017) was awarded a prestigious **2021 Mustafa Prize** in the study of science and technology, and for the study of Weyl fermion semimetals, in particular.

2021 New Miller Fellows Retreat



Recent Publications

Jill Banfield (Miller Professor 2006-2007), **Adair Borges** (Miller Fellow 2020-2022) & **Jennifer Doudna** (Miller Senior Fellow 2017) are co-authors of the paper, "*Species- and site-specific genome editing in complex bacterial communities*" published in the journal *Nature Microbiology*.

William Boos (Miller Professor 2021) is a co-author of the article "*Mechanical forcing of the North American monsoon by orography*" published in *Nature*.

Feng Wang (Miller Fellow 2005-2008, Miller Professor 2021-2022) is a co-author of the papers, "*Imaging two-dimensional generalized Wigner crystals*" & "*Measuring phonon dispersion at an interface*" published in *Nature*.

Norman Yao (Miller Fellow 2014-2017) and colleagues at QuTech reported the creation of a many-body localized discrete time crystal that lasted for about eight seconds in a paper published in the journal *Science*.

Tanja Cuk (Miller Fellow 2007-2010) is a co-author of the paper "*Free energy difference to create the M-OH* intermediate of the oxygen evolution reaction by time-resolved optical spectroscopy*" published in *Nature Materials*.

John Hartwig (Visiting Miller Professor 2009) is one of four senior authors of the study, a collaboration between synthetic chemists and synthetic biologists at the University of California, Berkeley, and Lawrence Berkeley National Laboratory, that led to engineering bacteria that can make a molecule that, until now, could only be synthesized in a laboratory. The findings were published online in the journal *Nature Chemistry*.



Gifts to the Miller Institute

The Miller Institute gratefully acknowledges the following contributors to the Miller Institute programs in 2021. With your generosity, the Miller Institute is able to continue to support basic research in science at UC Berkeley.

Kathryn A. Day Miller Postdoctoral Fellowship Fund

The Kathryn A. Day Miller Postdoctoral Fellowship was established with a generous gift by Nobel Laureate Professor Randy Schekman and Professor Sabeeha Merchant to honor Kathy Day, who served as the Chief Administrative Officer at the Miller Institute for Basic Research in Science from 1989 - 2019. The purpose of the Fund is to provide an annual stipend, benefits and a research fund to a postdoctoral researcher at the Miller Institute who has demonstrated efforts towards community building and outreach in support of science.

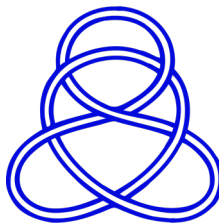
Anonymous (4)
Rachel Akeson
Steven Block - In Memory of Arthur Ashkin
Justin Brown
Roland Burgmann (2)
Mary (Beth) Burnside
John Clarke
Kathy Day (2) - In Memory of Vaughan Jones (1)
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Michael Manga & Susan Storch
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Scott Laurence Miller - In Memory of Judey Miller
Vijay Pande
Phillip Peebles
Eliot Quataert
Talat Rahman
Jasper Rine
Martin Scharlemann
Montgomery Slatkin
Terence Speed
Jesse Thaler (2)
Jeremy Thorner - In Honor of Kathy Day
Rebecca Bishop White - In Memory of Irma Reyna
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Miller Institute for Basic Research in Science General Fund

The Miller Institute for Basic Research in Science is dedicated to the encouragement of creative thought and the conduct of research and investigation in the field of pure science. Contributions to this fund will support the four programs of the Miller Institute: the Miller Research Fellowship, the Miller Professorship, the Visiting Miller Professorship, and the Miller Senior Fellowship.

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Steven & Diana Brenner
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Daniel Ibarra
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Norman Yao

Miller Fellowship Program Development Fund

The Miller Fellowship Program Development Fund provides an annual stipend, benefits, and research support to young researchers at Berkeley. The program gives researchers the chance to explore ideas in a stimulating and supportive environment.

Dan-Virgil Voiculescu

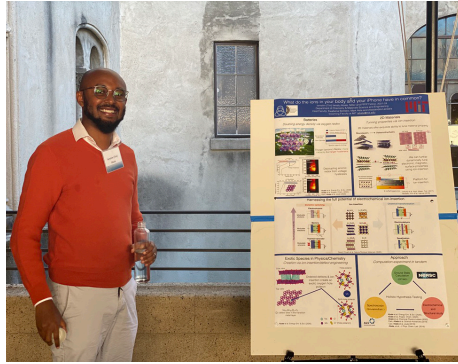


2021 Miller Institute Mementos

First Annual Fall Retreat



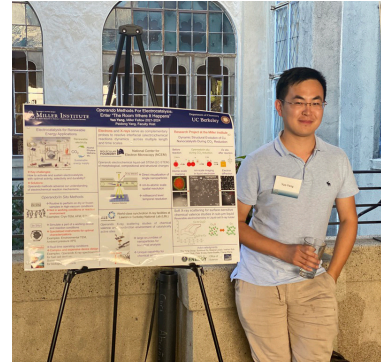
Miller Fellow Anna Ho & Miller Senior Fellow Alex Filippenko



Miller Fellow Iwnetim Abate



Former Miller Senior Fellow Randy Schekman & Symposium Chair Michael Manga



Miller Fellow Yao Yang

Fall Retreat Speakers: Ron Cohen, Michael Jordan, Nicole King & Norman Yao



Former Miller Fellow Yong Zheng & Miller Fellow Aaron Joiner

Miller members Naomi Latorraca, William Boos, Antoine Koehl, Yi Zhang & Ellen Vitercik on a post-retreat hike at the Point Reyes National Seashore



Annual Fall Dinner 2021



Former Miller Senior Fellow Raymond Jeanloz, Executive Committee Member Chung-Pei Ma & Chancellor Carol Christ



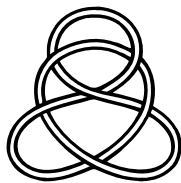
Executive Committee member Yun Song & Advisory Board member Scott Edwards



Miller Fellows Veronika Sunko (left) & Ekta Patel (right) with guests Petar Petrov & Nick Miller



Miller Fellows Nayeli Rodriguez Briones & Danqing Wang



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2021 Miller Picnic



Group photo of Miller members at the Fall picnic

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Group photo of Miller Fellows on a post-retreat hike at the Point Reyes National Seashore

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