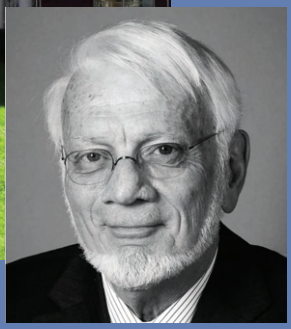
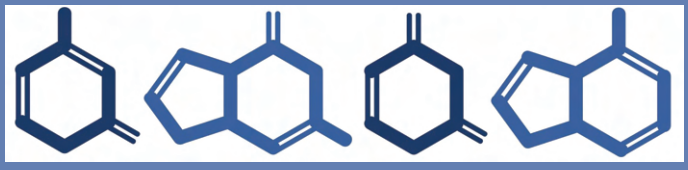
Tom and Joan Steitz RNA Fellows

Yale Center for RNA Science and Medicine





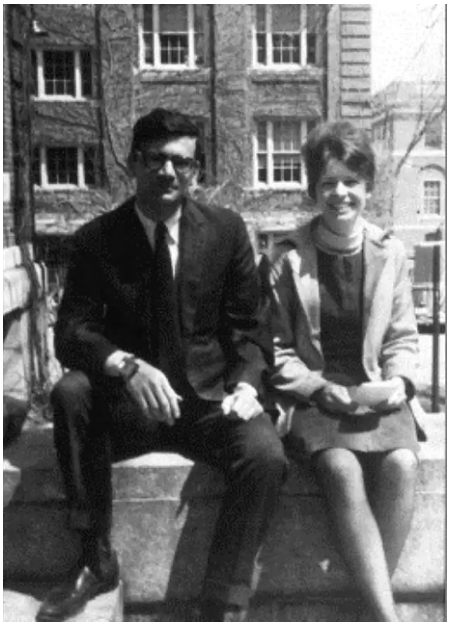
2023-2024

Background

Joan (left) and Tom Steitz (right)

Joan A. Steitz, PhD, Sterling Professor of Molecular Biophysics and Biochemistry and alumna investigator of the Howard Hughes Medical Institute, donated her 2021 Wolf Prize to the Yale Center for RNA Science and Medicine. Her contribution led to the founding of the Tom and Joan Steitz RNA Fellows Program, which will honor Yale undergraduate, graduate, and postdoctoral researchers who show promise as future leaders in the field of RNA biology. The goal of the fellowship is to create an intergenerational community of RNA scholars that will help foster the scientific excellence, career opportunities, and leadership potential of each awardee through alumni, peer, and faculty support. The program is inspired by Joan Steitz's extraordinary history of mentorship.

Award



Tom (left) and Joan Steitz (right)

Each class of fellows will be recognized in February at a special *Tom and Joan Steitz RNA Fellows Dinner*. The fellows' mentors will also be invited. The RNA Fellows from previous years will be invited back each year for the Fellows Dinner honoring the next class of Tom and Joan Steitz RNA Fellows, thus growing the community of fellows. In addition, the winning essays of each fellow in the new class will be published in the *Tom and Joan Steitz RNA Fellows Bulletin* disseminated annually to the community of fellows. The essays will also be publicly available on the Yale Center for RNA Science and Medicine website on the Fellows Program page.

The Yale Center for RNA Science and Medicine, which administers the Tom and Joan Steitz RNA Fellows Program, is led by director Karla M Neugebauer, PhD.

These are extremely exciting times in RNA science. Over the past few decades, researchers have discovered that RNA molecules are critical players in multiple areas of biology and biomedical science. Most recently, scientists have discovered that much of our genome, long believed to be silent, actually codes for RNA molecules. Although we are just beginning to realize how much remains to be understood about these RNAs and their functions, many of the new RNAs have already emerged as both critical diagnostics and key therapeutic targets in disease.

The mission of the Yale Center for RNA Science and Medicine is to build upon Yale's tremendous strengths in RNA biology, to foster interdisciplinary interactions and to apply our collective knowledge to understand disease processes and discover new treatments. Our members come from more than a dozen Yale departments and their laboratories are located at all three Yale campuses.

Center-sponsored events include our annual retreat, RNA Club, workshops, and seminars by leading researchers. Our goals are to foster a sense of community and to encourage collaborations, both between our world-class RNA scientists and with the many other extraordinary scientists and clinicians here at Yale.
If you should have any questions about the RNA Center's mission, or have ideas for events you would like to see sponsored by the Center, please contact our leadership.

Tom and Joan Steitz RNA Fellows Class of 2023

Class of 2023 gathers for lunch. Clockwise from left: Annsea Park, Amer Balabaki, Kyrillos Abdallah, Sudheesh Parambil, Gaëlle Talross, Emily Sutton.

Kyrillos Abdallah

Steitz Fellows Class of 2023
Graduate Student,
Laboratory of Wendy Gilbert

Eukaryotic translation initiation is the rate-limiting step for protein synthesis, determining the number of protein molecules synthesized from a single messenger RNA (mRNA). Canonical translation initiation relies on recognition of the 5′ N7-methylguanosine cap by eukaryotic initiation factors (eIFs), which subsequently recruit the ribosome to the mRNA. However, cellular and viral mRNAs use diverse, non-canonical mechanisms to initiate translation. Intriguingly, some well-structured RNA elements can recruit the translation initiation machinery directly, without using a 5′ cap. Such internal ribosome entry sites (IRESs) have been well characterized in certain viral untranslated regions (UTRs) including at atomic resolution; by contrast, the very existence of IRESs in human 5′ UTRs remains controversial. My project, identifying and dissecting mechanisms of internal translation initiation in human 5′ UTRs, will provide insight into fundamental biology by illuminating alternative mechanisms of ribosome recruitment that are likely to be important for regulating gene expression in human cells.

Since joining the Gilbert lab in May 2022, I have successfully adapted our novel high-throughput technique, Direct Analysis of Ribosome Targeting (DART), to investigate cap-independent translation initiation in human 5′ UTRs. In collaboration with the Thoreen lab, I have generated a pool of thousands of human 5′ UTRs embedded in circular RNAs. Without a free 5′ end, any ribosome recruitment to circular RNAs must proceed through internal initiation. Using DART, I aim to comprehensively identify putative IRESs in human UTRs and delineate the RNA elements required for IRES activity. I will then elucidate their mechanisms using low-throughput biochemistry. Importantly, my approach specifically examines the initiation stage of translation thereby overcoming the limitations of previous studies which could not disentangle effects on translation initiation from other cellular processes.

The significance of this project extends beyond the bench. Circular RNAs are appealing as a “next-generation” RNA therapeutic. Because these RNAs lack 5′ and 3′ ends, they evade host exonucleases, escaping many RNA degradation pathways and innate immune responses in cells. However, internal initiation is generally less efficient as compared to cap-dependent translation initiation. By identifying and characterizing novel sequences with high internal translation initiation in circular RNAs, I aim to engineer circular RNAs that sustain high levels of therapeutic protein production in patients.

Sudheesh Allikka Parambil

The recent SARS-CoV2 outbreak demonstrates that viruses remain a threat to human health. Although vaccines can be developed retroactively, forecasting the next outbreak is challenging. Alternatively, we could aim to leverage the inherent cell-autonomous mechanisms of human cells to broadly boost our anti-viral resistance. This approach is crucial for stem cells, because, unlike differentiated cells, they lack the essential anti-viral interferons to co-ordinate the cell-based innate immune system.

Steitz Fellows Class of 2023
Postdoctoral Associate, Laboratory of Josien van Wolfswinkel

I use the planarian Schmidtea mediterranea to investigate nucleic acid sensors and non-coding RNAs in anti-viral defense in stem cells. Planarians have an abundance of long-lived adult stem cells that are crucial for homeostasis. Absence of cell-based immune systems makes planarians a perfect model to study cell-autonomous immunity. My preliminary research demonstrates that dsRNA responders including RIG-I-like receptors (RLRs) and several components of RNAi-mediated silencing display increased expression upon viral challenges. Remarkably, I also identified a piRNA factor in this response, and knockdown of the identified RNAi and piRNA factors leads to increased viral load in the animals. This indicates the unexpected collaboration between these two small RNA pathways in stem cells for viral defense. I am using biochemical approaches to identify further factors in this pathway.

Currently, there is only one known planarian virus, so I developed an in-house method to isolate and identify viruses from S. mediterranea. Interestingly, most of the families identified were RNA viruses. We will study the differences in responses against dsDNA and RNA viruses as they threaten the cellular physiology differently. Moreover, we plan to leverage this information to develop a planarian transgenics system, a current limitation in the field. I have a longstanding interest in RNA biology. During my master's degree, I developed a molecular diagnostic technique for detecting the endemic tropical disease (leptospirosis) outside of a lab setting, igniting my interest in research. This helped hospitals to diagnose this condition in remote areas. I currently develop a low-cost nucleic acid-based diagnosis kit for multiple zoonotic diseases. For my graduate work, I investigated how nuclear phosphatidylinositol-phosphate (PIP) lipid signaling controls mRNA 3'-end processing. We laid the groundwork for future RNA therapies by disclosing a novel role for alternative polyadenylation and PIP signaling in controlling translation in pathological states such as hypertrophy, and metastasis.
In summary, RNA has immense therapeutic potential, and I hope to eventually run an RNA research lab and continue to translate my working knowledge of RNA into low-cost diagnostics and treatments for current and future outbreaks.

Amer Balabaki

Steitz Fellows Class of 2023
Undergraduate, Laboratory of Joan Steitz
Now a graduate student at MIT

Over the course of evolution, molecular adaptations within RNA structures allowed for functional versatility. Cis-acting RNA elements are secondary structures implicated in RNA metabolism, stability, translation, and processing. One cis-acting RNA element that is present in viruses and retrotransposons is the element for nuclear expression (ENE). First discovered in the PAN RNA of Kaposi's sarcoma-associated herpesvirus, ENEs are elements that harbor U-rich internal loops flanked by short helices. Found in the 3'-proximal regions of transcripts, ENEs function by sequestering poly(A) tails into major-groove triple helices. This interaction protects poly(A) tails from rapid deadenylation-dependent decay, which is often the first step involved in eukaryotic RNA degradation. With that said, transcripts containing these ENEs have a stability advantage that is modulated through evolutionary pressures.

As part of the research I am doing in the lab of Dr. Joan Steitz, bioinformatic analyses of the genomes of vertebrates have revealed that some LINE retrotransposons are enriched in ENE sequences. To test whether these identified ENEs bind poly(A) tails, I first sought to determine if binding can occur in-trans using electrophoretic mobility shift assays (EMSAs). This was done by binding radio-labeled oligo(A12) to in-vitro transcribed ENE RNAs. Indeed, the ENEs identified computationally do bind oligo(A12) in-vitro as expected. To probe the role of highly conserved residues in the stability of the triple helix, mutagenesis was performed, followed by EMSA. Surprisingly, some mutations increased the binding affinity of the ENE for oligo(A12), suggesting a novel role for these ENEs. Upon analysis of the literature, we hypothesize that these ENEs are involved in stabilizing poly-uridylated transcripts in the cytoplasm, as cytoplasmic uridylation of poly(A) transcripts is often a marker for RNA degradation. This could have many implications for science and society, as ENEs are rarely studied in cytoplasmic environments. This could allow us to understand how retroelements and viruses passing through the cytoplasm avoid RNA degradation. As a result, one could theoretically target those elements using anti-morpholino oligos to destabilize the transcript and prevent viral progression. This research can also be utilized to develop better mRNA vaccines to fight pandemics, as therapeutic mRNAs can be stabilized by adding ENEs. This strategy should allow for the production of more robust immune responses upon vaccination. Thus, studying ENEs is one example of how basic advances in RNA biology can lead to improvements in therapeutics.

Kevin Chen

Steitz Fellows Class of 2023
Undergraduate, Laboratory
of Nadya Dimitrova

Not too long ago, most of the human genome was considered "junk" DNA – that is, DNA without protein-coding potential. We now know this to be false; instead, an intricate regulatory network exists within these noncoding regions. The center of this is noncoding RNA (ncRNA), whose novelty and ubiquitous appearance in numerous cellular pathways have garnered widespread attention. I am particularly interested in long ncRNA's (lncRNAs) role in p53 tumor suppressive pathway and cancer development. My research can be categorized into two themes: mechanistic dissection of p53-regulated lncRNAs (PR-lncRNAs), and discovery of novel coding genes within the p53-regulatory network.

My fascination with lncRNAs started with Xist, a noncoding transcript that epigenetically silences chromatin for sex determination. Discovered in the 1990s, Xist is one of the most well-characterized lncRNAs. Yet, its mechanism of action largely remains a mystery. The diversity of lncRNAs has made it challenging to evaluate their roles in normal and diseased contexts, including cancer. My research focused on implementing gene editing technology to functionally characterize PR-lncRNAs. I developed a paired-guide RNA CRISPR/Cas9 deletion strategy that allows me to dissect elements within Pvt1, a PR-lncRNA that regulates the proto-oncogene Myc, and investigate their individual functions. This work unveiled key functional elements that regulate Myc and expanded our understanding of the Pvt1 locus and lncRNA activity in cancer. This work also validated the use of CRISPR/Cas9-mediated deletions in lncRNA studies, contributing to a growing list of tools for lncRNA biologists.

Recently, my interest with space exploration gave me the opportunity to investigate RNA from a unique perspective. Through the NASA CTSpace Consortium, I am currently studying novel coding genes within the ionizing radiation (IR) induced p53 response. Advancements in sequencing technology have revealed interesting coding potential within erroneously annotated lncRNAs. Therefore, my current work aims to discover, validate, and characterize novel proteins in the IR-response pathway that were once believed to be encoded by lncRNAs. My work will re-evaluate the functions of lncRNAs and further our knowledge of the p53 network in outer space. This intersection between RNA biology and space biology may bring about new solutions in improving human health.

My goal is to harness the potential that RNA has and utilize them to advance therapeutics for cancer and other diseases. Ultimately, I strive to pursue a career where I can apply my research in treating patients both on Earth and in space.

Annsea Park

Steitz Fellows Class of 2023
MD/PhD Student,
Laboratory of Akiko Iwasaki

Greater than 75% of the human genome is pervasively transcribed, although only 1.5% encodes proteins. Non-coding RNAs (ncRNAs) are major functional and regulatory units of the non-coding part of the genome, yet remain under-explored. With increasing evidence for their perturbations in human disease pathogenesis, the remarkable variety of functions that ncRNAs can accomplish present particularly exciting areas for research.

Dissecting the mechanisms by which RNA molecules are translated into proteins in Dr. Cate's lab at UC-Berkeley as an undergraduate student, I became fascinated by the versatile functions of RNA molecules fundamental to human health. I also wanted to integrate my research into understanding human disease and patient care. To this end, I exclusively applied to MD-PhD programs and chose Yale because of its strength in RNA biology and collaborative research community. Indeed, interdisciplinary collaboration and the supportive RNA community have been key aspects of my training at Yale.

My interest in RNA biology naturally led me to Dr. Steitz and a novel class of ncRNAs that her lab had just discovered. Relatively little is known about these RNA species, called Downstream-of-Gene (DoG) RNAs, other than the fact that they are expressed during various stress conditions such as viral infections and cancer. During my PhD, I wished to understand the function of these RNAs not only at a molecular but also at a systems level, especially in the context of human immune response. Systematically investigating their expression across human tissues, I discovered elevated levels of DoG RNAs in cerebellar neurons and described their potential role in suppressing the innate immune response by acting on retrotransposon transcripts. These findings suggest that DoG RNAs represent a strategy to prevent inflammation and cell death in non-replicating cell types and that their insufficient expression contributes to aberrant or hyperactive immune response.

The clinical and research training I have received at Yale continue to fuel my career goal as a physician-scientist in the fields of RNA biology, immunology, and medicine. I hope to synergize my clinical and research interests as an academic investigator dedicated to investigating mechanisms that drive autoimmunity, with a keen interest on the contributions of non-coding and retrotransposon-derived RNAs. Through my perseverance and commitment to uncovering RNA biology to better understand human disease, and the support of an exceptional community of RNA scholars and dedicated mentors, I will strive to become a physician-scientist who melds together her passions for medicine and research.

Emily Sutton

Steitz Fellows Class of 2023
Postdoctoral Associate,
Laboratory of Susan Baserga

I have been fascinated by RNA since I first learned about the milieu of biomolecules essential for cell survival and proliferation. My research focuses on how ribosomes – the molecular machines that translate messenger RNA (mRNA) into protein – are made. Ribosomes have one small and one large subunit, each of which are comprised of ribosomal proteins and ribosomal RNA (rRNA). That these essential molecular machines translate one type of RNA while being comprised of another is a remarkable testament to the importance of RNA biology to all functions of living organisms.

In eukaryotic cells, ribosomes are made in the nucleolus, a membraneless organelle within the nucleus. During my graduate work, I studied chemotherapeutic platinum compounds that localize to the nucleolus and how they interfere with the creation of ribosomes. These anticancer drugs were discovered decades ago and are still widely used today, however they were only recently found to inhibit ribosome biogenesis. My research identified highly specific structural properties for platinum compounds to interfere in this process.

My current postdoctoral research in the lab of Dr. Susan Baserga seeks to address broad questions about the regulation of ribosome biogenesis. Aberrations in ribosome biogenesis and the nucleolus are strongly associated with disease. Elevated ribosome biogenesis is implicated in some cancers, and changes in nucleolar morphology have been associated with cancer for over a century. Ribosomopathies are genetic developmental diseases, often severe, driven by abnormal ribosome biogenesis. Despite the importance of ribosomes, there is much still to learn about how they are generated in the cell.

A critical line of inquiry is how changes in ribosome biogenesis regulation can lead to disease outcomes. Another open question driving my current research is how ribosome biogenesis is regulated by other important cellular processes, organelles, and pathways. I focus on proteins newly identified via screens to be regulators of ribosome biogenesis, especially those with disease relevancy and the potential to uncover previously undescribed relationships between ribosome biogenesis and other processes. For example, I am investigating how a protein primarily involved in transport from the Golgi apparatus to the endoplasmic reticulum seems to be regulating nucleolar ribosome biogenesis in human cells. Explaining these surprising connections will deepen our understanding of how ribosomes are made, with tremendous potential to provide insights into both basic RNA biology and human health.

Gaëlle Talross

Steitz Fellows Class of 2023
Postdoctoral Associate,
Laboratory of John Carlson

Many aspects of RNA biology shape neural function: (i) The nervous system displays unmatched levels of transcriptional and post-transcriptional regulation, events crucial for neuronal identity and plasticity. (ii) Timing and localization of mRNA translation impact the dynamic of neuronal response. (iii) Numerous classes of RNA perform critical roles in neurons: non-coding (nc)RNAs, renowned for their multifaceted nature, are ideally suited for roles in modulation of neuronal activity and evolution. Several neurological disorders are associated with the dysregulation of these processes, highlighting the importance of mRNA tailoring and ncRNAs functions. Their investigation in the nervous system is a challenging frontier that requires exploration of uncharted territories.

In the Carlson laboratory, I examine such uncharted territory: neuronal RNA in Drosophila chemosensory systems. These well-characterized and highly adaptive neuronal systems are numerically simple yet drive multiple complex behaviors. With powerful genetic tools and low genetic redundancy, Drosophila offers an ideal opportunity for unparalleled investigation of mRNA tailoring and ncRNAs.

I began my studies by generating transcriptomes for two chemosensory organs, the antenna (olfaction) and the labellum (taste), and searched for non-canonical transcript variants of chemosensory genes and long ncRNAs. Such analyses divulged 3 layers of complexity to chemosensation:

1. Drosophila chemosensory genes encode more transcript variants than previously appreciated. RNA-Seq revealed evidence for alternative transcription start/termination sites and rare splicing events of chemoreceptor transcripts. Xueying Shang leads investigations into the role(s) of such events in neuronal adaptation.
2. Stop codon read-through prevails in chemoreceptor genes. Analysis of chemoreceptor transcripts revealed that a receptor for salt taste harbors an unexpected conserved premature termination codon (PTC). Genetic experiments showed that synthesis of a functional receptor requires PTC read-through.
3. Olfactory receptor neurons express unique lncRNAs. By combining bulk and single-nucleus RNA-Seq analyses, we generated the first database of antennal lncRNAs and an unprecedented lncRNA-to-neuron map. Intriguingly, a number of these lncRNAs are species-specific and expressed primarily in pheromone-sensitive neurons, inviting us to explore their role in species recognition.

My work provides the foundation for identifying mRNA tailoring and lncRNAs characteristic to the adaptation to new environments/internal states. I identified one of such RNAs: ANRUS, ANtennal RNA Upregulated by Starvation. ANRUS shows numerous ncRNA characteristics, yet it encodes 2 micropeptides. I am investigating their roles in modulating neuronal activity of fasted animals.

Overall, my research offers new biological insights into chemosensation and introduces a new platform to investigate the vast impact of RNA biology in neurons.

Tom and Joan Steitz RNA Fellows Class of 2024

Zhiliang Bai

Steitz Fellows Class of 2024
Postdoctoral Associate,
Laboratory of Rong Fan

Spatial transcriptomics has emerged as a powerful tool for dissecting cellular heterogeneities within their native tissue context but as of today is predominantly confined to mRNA examination. Yet, the life of RNA molecules is multifaceted and dynamic, requiring spatial profiling of different RNA species throughout the life cycle to delve into the intricate RNA biology in complex tissues. Formalin-fixed paraffin-embedded (FFPE) tissues are essential in clinical practice, being the backbone of all human biopsy diagnoses. The capacity to spatially explore RNA biology in FFPE tissues holds transformative potential for pathology research. Nevertheless, the RNA within these samples is susceptible to degradation during the paraffin-embedding process and may further experience heightened degradation under suboptimal storage conditions. Additionally, RNA may undergo chemical modifications, resulting in fragmentation or resistance to the enzymatic reactions required for sequencing. The loss of poly-A tails introduces another layer of complexity, restricting the use of oligo-dT primed reverse transcription.

In Dr. Rong Fan's lab, I lead the development of Patho-DBiT, an innovative technology tailored for spatial whole transcriptome sequencing meticulously crafted to address the distinctive challenges of clinically archived FFPE tissues. Patho-DBiT integrates in situ polyadenylation, deterministic barcoding in tissue using microfluidic chips, and computational innovations to navigate and decode RNA regulations inherent in FFPE samples. Capitalizing on the inhibitory impact of formalin fixation on endogenous endonuclease activity and RNA fragmentation naturally occurring in FFPE specimens, Patho-DBiT even outperforms fresh frozen tissue spatial transcriptomics and further allows for the profiling of a broad spectrum of RNA species.

Patho-DBiT permits spatial co-profiling of gene expression and alternative splicing, unveiling region-specific isoforms in the mouse brain. High-sensitivity transcriptomics is constructed from 5-year archived T-cell lymphoma tissues, with cross-validation conducted using super-resolution spatial phenotyping technology (CODEX). Furthermore, genome-scale single nucleotide RNA variants are captured to autonomously distinguish malignant from non-malignant cells in B-cell lymphomas. Patho-DBiT also enables spatially resolved co-profiling of large and small RNAs, facilitating the analysis of a microRNA-mRNA regulatory network within clinical biopsies and elucidating their roles in tumorigenesis. With superior intronic read capture efficiency, Patho-DBiT spatially maps RNA splicing dynamics associated with the developmental trajectory of tumor cells. High- resolution Patho-DBiT with a 10-μm spot size reveals the heterogeneities of human lymphomas within a neighborhood and traces the spatiotemporal molecular kinetics driving tumor progression at the cellular level. Patho-DBiT represents a first-of-its-kind technology, enabling the spatial exploration of rich RNA biology in FFPE tissues to aid pathology diagnosis.

Hannah Barsouk

Steitz Fellows Class of 2024
Undergraduate, Laboratory of
Ronald Breaker

In a version of life derived from RNA, RNA molecules took on the catalytic or regulatory functions typically performed by proteins in modern biology. Long non-coding RNAs (ncRNAs) maintained in bacteria are believed to be remnants of this 'RNA World,' and as such often participate in conserved and fundamental biological processes. As a high school student, I was given the opportunity through a public-school outreach program to conduct research at the University of Pittsburgh. Over my three years studying conserved energy pathways in yeast, I fell in love with ancient biology and the RNA molecules which often lie at its core. One year, I even dressed up as an essential regulatory process, 'alternative splicing,' for Halloween.

In October of 2021, I joined the Breaker Lab to further explore my interests in the RNA World and molecular evolution of life. Alongside incredible mentors, I have elucidated novel roles that non-coding RNAs likely played in regulating central carbon metabolism or metal homeostasis in early life. My current project concerns one of the largest and most widespread classes of bacteria ncRNAs whose exact biochemical function remains unknown: the Ornate, Large, Extremophilic (OLE) RNA. OLE RNAs form a ribonucleoprotein (RNP) complex which is required for cells to adapt to diverse environmental stresses such non-glucose carbon sources or excessive magnesium concentrations. As such, the Breaker Lab has recently theorized that the OLE RNP complex acts as a master regulator of cell growth in bacteria.

Previous genetic screens involving the OLE RNA suggest cells without a functional OLE RNP complex experience more ribosome stalling events. This observation motivated my hypothesis that the OLE RNA controls cell growth under stress by affecting ribosome stalling. Thanks to Tom Steitz's careful work characterizing the mechanisms of action of several ribosome-targeting antibiotics, I assessed the effect of inducing ribosome-stalling in cells lacking the OLE RNP complex. Surprisingly, I found that in the absence of OLE RNA, cells possessed increased resistance to ribosome-stalling antibiotics.

The precedent for the role of ncRNAs in gene regulation and stress response makes these macromolecules a largely unexplored avenue to antibiotic resistance in bacteria. As I pursue a Ph.D. in biochemistry, I hope to continue to study rare and ancient RNAs. Investigation into classes like OLE RNA may uncover common biochemical phenomenon which underly paths to stress adaptation and antibiotic resistance across bacterial species.

Moreen Ng

Steitz Fellows Class of 2024
Undergraduate, Laboratory
of Joan Steitz

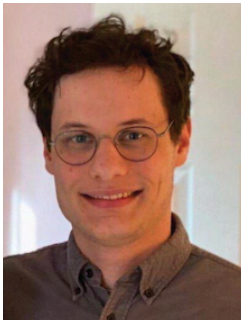
Prior to the discovery of non-coding RNAs (ncRNAs), it was assumed that non protein-coding genetic material did not possess a functional purpose. However, decades of scientific research unveiled diverse classes of ncRNAs that are crucial for numerous molecular pathways in prokaryotes, eukaryotes, and even in viruses. As viruses have a constrained genome, any viral transcriptional output likely has a function to ensure successful infection and proliferation in the host. Introduced to the intricacies of ncRNAs in my biochemistry courses, I was fascinated by ncRNAs' therapeutic potential and the enduring mysteries on their function, which has led me to the robust RNA community at Yale where I investigate the role of viral ncRNAs in Epstein-Barr virus (EBV) in Dr. Steitz's lab.

EBV is a double-stranded DNA tumor virus that infects B-cells with a widespread influence, having infected over 90% worldwide. As an oncogenic herpesvirus, EBV entails a lifelong infection with increased risks of developing Burkitt's lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma. Interestingly, EBV encodes various ncRNAs, even during latency when gene expression is minimal. Most notably, EBV encodes an abundance of ncRNA EBER2, which regulates the expression of latent genes necessary for B-cell transformation and is crucial to EBV malignancy.

As part of my research in the Steitz lab, I investigate EBER2's role in maintaining latency and inducing lytic replication, which remains largely unknown. Specifically, I seek to understand the composition and function of the ribonucleoprotein (RNP) complex consisting of EBER2, host transcription factor PAX5, and the terminal repeat (TR) region of the EBV genome. EBER2 and host transcription factor PAX5, which is a major B-cell regulator, was discovered to indirectly colocalize to the TR region. As in-vivo crosslinking and high-throughput sequencing methods identified a novel non-coding RNA transcript as the potential intermediary that recruits EBER2 and PAX5 to the TR region of the viral genome, my current work seeks to characterize this novel transcript. Recently, I have successfully identified the start sites of several alternatively spliced variants of this novel ncRNA and aim to further investigate how particular spliced variants may contribute to the RNP complex's regulation of EBV life cycle.

The prospect of my research synergizes both scientific and societal implications. Further understanding of how ncRNAs contribute to EBV regulation and malignant growth will expand our growing knowledge of ncRNAs' roles in viruses, while elucidating potential antiviral strategies that could alleviate EBV's global impact and oncogenic ramifications.

Leo Schärfen



Steitz Fellows Class of 2024
Graduate Student, Laboratory of Karla Neugebauer

Through base pairing, cellular RNAs adopt unique functional structures that determine and regulate their catalytic, structural, and coding potential. Understanding base pairing is therefore crucial to harnessing the full potential of RNA, e.g. for therapeutic purposes. As such, one of the most fundamental questions in RNA biology remains mostly open: Base pairing within cells is currently hard to reliably predict based on sequence alone.

When I started my PhD in Karla Neugebauer's lab, I realized that nevertheless, many researchers report that the predicted stability of the most stable RNA structure from the ensemble of all possible structures (MFE structure) correlates with some experimental read-out. Translation, splicing, degradation, localization – all correlated with RNA structure. Rarely do we find molecular mechanisms behind those correlations. I hypothesize that our understanding might be limited by a prevailing model of cellular RNA that is starting to be replaced. In an RNA version of the circular reasoning fallacy, we often depict RNA as adopting one defined structure, since we tend to study the structure of RNAs with defined structural states; those are easier to solve. Most RNA species however, especially mRNAs with evolutionary pressure on protein rather than RNA structure, are more likely to co-exist in many distinct base pairing patterns with different stabilities. Therefore, what correlates with translation, localization, etc. of an RNA may not be the stability of one of its predicted structures, but rather its *base pairing potential*. This might often be well- approximated by the stability of one structure from the ensemble, but crucially, the concept behind the measured variables is different and might affect our interpretation.

In my current research, I try to approach this issue by studying how base pairs first arise. By developing a new method that simultaneously detects pairing status and RNA polymerase (Pol) position on individual nascent transcripts, I show that RNA can base pair directly after exit from Pol. I show that in pre-rRNA, dynamic local base pairing predominates leading to structures that differ vastly from mature rRNA, which relies on long-range interactions that cannot form co- transcriptionally. In contrast, nascent pre-mRNA appears very similar to mRNA, suggesting that mRNA structures are dominated by small-scale local interactions which might be dictated by the process of transcription itself.

In my future academic work, I would like to continue adding to conceptual models for mRNA structure in a cellular context that incorporate RNA's dynamic, metastable nature more explicitly, hopefully allowing us to explain more of our correlations with molecular mechanisms.

Ethan Strayer

Steitz Fellows Class of 2024
Graduate Student, Laboratory
of Antonio Giraldez

Life requires spatial and temporal control of protein production. The protein output of a given transcript reflects the integration of translation and mRNA stability. My work seeks to understand how cis-regulatory elements encoded in the untranslated regions of mRNAs regulate translation. To this end, I have developed NaP-TRAP, Nascent Peptide Translating Ribosome Affinity Purification, a Massively Parallel Reporter Assay (MPRA) that measures translation through the immunocapture of the epitope tagged-nascent chain complexes of reporter mRNAs. Using this assay, I have investigated the regulatory potential of endogenous and synthetic UTRs in the developing zebrafish embryo and human cell lines.

Given that initiation is the rate limiting step of translation, my worked has focused predominately on characterizing 5' UTRs. In canonical eukaryotic translation initiation, the small ribosomal subunit is recruited to the 5' UTR by eukaryotic initiation factors following cap-recognition. The subunit then scans along the 5' UTR until it encounters a start codon, resulting in the subsequent recruitment of the large ribosomal subunit and the initiation of translation elongation. Cis-regulatory elements in the 5' UTR can affect each step of this process. Using NaP-TRAP, I have identified universal activators and repressors as well as developmentally dynamic regulators of translation in the 5' UTR. Yet this experience has left me with more questions than answers. For example, the reporter mRNAs that I tested were fragments of endogenous 5' UTRs, yet some of the reporters exhibited extremely low translation values. This raises an interesting question: how are endogenous transcripts with multiple of the repressive elements in their 5' UTRs translated at all? Perhaps these mRNAs exhibit cell-specific translation activation or serve a functional role beyond the protein for which they encode.

As I think about the future, the potential of mRNA therapeutics excites me. mRNA provides a highly customizable treatment modality without the risks and expense associated with genomic editing. Further, the regulatory potential of RNA is limitless. There are more potential sequence combinations in a 150 nucleotide segment of RNA than there are particles in the known universe. This expanse presents an exciting opportunity for technological innovation. Within the relatively small portion of the sequence space that life has sampled, there exists incredibly intricate modes of regulation. Advances in MPRAs, sequencing, and machine learning will enable us to explore this massive space in a systematic and rational manner. Who knows what we will find.

Lucille Tsao

Steitz Fellows Class of 2024
Graduate Student,
Laboratory of Anna Marie Pyle

My fascination with RNA structure began during college when I first performed an in silico design of RNA-like motifs that could disrupt the frame-shifting pseudoknot of SARS-CoV-2. This experience oriented me towards exploring the crucial roles RNA structures play in translation, replication, and immune evasion in viruses. Flaviviruses, which includes Dengue, Zika, and West Nile, are viruses that possesses a vital RNA structure. These RNA viruses lead to severe diseases, such as hepatitis, encephalitis, and congenital abnormalities. With over 400 million annual infections globally, there are no pan-flaviviral therapeutics to address this great global health threat. To create effective RNA targeted therapeutics, we need to fill the critical gap in our understanding of RNA structures in viral pathogenesis and how to leverage them.

In Dr. Anna Pyle's Lab, I focus on characterizing the architecture of the Flaviviral RNA genome. I realized that the development of an effective treatment first requires a complete structure of the subgenomic-flaviviral RNA (sfRNA). These highly conserved subgenomic RNAs are necessary for innate immune evasion, virus-induced apoptosis, and neuropathogenicity. Structurally, the sfRNA contains four pseudoknots that aid in its compaction, so I systematically interrogated the secondary structure for each pseudoknot using SHAPE-MaP. I identified a specific hierarchy in pseudoknot formation and found that the disruption of one pseudoknot could detrimentally affect sfRNA formation. Using Terbium-seq to further probe the tertiary structure, I found additional motifs that were conserved across multiple Flaviviruses, suggesting that these motifs could be potential targets for a pan-flaviviral therapeutic.

Currently, I am using cryo-EM to obtain a high-resolution structure of the sfRNA. I hope to use this structure to conclusively pinpoint potential drug binding targets. Leveraging this structure, I can further elucidate sfRNA cellular mechanisms and roles in flavivirus microevolution. I believe these structural insights will inform therapeutic development and allow us to predict future outbreaks. This work will also expand the repertoire of 3D viral RNA structures and revolutionize current understanding of structures influencing the viral lifecycle. My robust pipeline could be applied to other viral RNA systems, guiding the design of novel viral therapeutics. In line with my long-term scientific goals, I aspire to explore RNA structure- function relationships in human diseases in non-viral contexts, such as how long noncoding RNA structures may be involved in cancer. Through this structure-first approach, I aim to unravel principles of RNA structure and pave the way for the next generation of RNA therapeutics.

Denethi Wijegunawardana

Steitz Fellows Class of 2024
Graduate Student,
Laboratory of Junjie Guo

Within the diverse mechanisms at play in the expanding population of aging individuals, disrupted RNA processing has emerged as a prevalent theme, revealing a critical aspect of pathogenesis.

My journey into this fascinating realm began during my postgraduate research in Dr. Pallavi Gopal's lab at Yale, where I delved into the intricate world of mRNA and RNA-binding proteins (RBPs) in the context of neurodegenerative diseases. I became captivated by the complexities of ALS, characterized by the mislocalization and aggregation of TDP-43, an RBP crucial for post-transcriptional RNA processing. My research uncovered complex interactions between TDP-43 and Ataxin-2, a polyglutamine (polyQ)-containing RBP also implicated in ALS. I found that Ataxin-2 polyQ expansions aberrantly sequester TDP-43, disrupt dynamic anterograde transport of TDP-43 ribonucleoprotein (RNP) condensates along axons, and increase their propensity to transform into pathologic aggregates. I discovered that translation of mRNAs critical for axonal and cytoskeletal integrity was suppressed in neurons expressing Ataxin-2 with polyQ expansions, providing mechanistic insights into the distal axonopathy associated with neurodegeneration.

Now, as a graduate student in Dr. Junjie Guo's lab, I continue fueling my passion for RNA research. Working with Suzhou Yang, another graduate student, we discovered the mechanism by which an intronic nucleotide repeat expansion (NRE) in the *C9ORF72* gene may encode toxic dipeptide repeat proteins in ALS and frontotemporal dementia (FTD). By capturing and sequencing NRE-containing RNAs from patient-derived cells, we elucidated that the *C9ORF72* NRE is exonized by the usage of downstream 5′ splice sites and exported from the nucleus in a variety of aberrantly spliced mRNA isoforms. We also found that *C9ORF72* aberrant splicing was substantially elevated in both in vitro differentiated motor neurons and post-mortem brain tissues. Following up on this work, I aim to identify common RNA risk factors that may unify ALS/FTD and other age-related neurodegenerative diseases, using directly differentiated neurons and organoid models that can preserve the epigenetic age of individuals. My goal is to understand the role of mRNA (mis)splicing in both convergent and divergent disease phenotypes.

Through the years my passion for RNA research has only intensified. Becoming part of the Joan and Tom Steitz RNA Fellows Program would serve as a catalyst for my future discoveries and broaden my horizon as an RNA biologist. By unraveling the molecular intricacies of RNA dysregulation, I aim to lay the groundwork for future development of targeted therapeutics and effective interventions in the relentless battle against neurodegenerative diseases.

Ningning Zhang

Steitz Fellows Class of 2024
Postdoctoral Associate,
Laboratory of Jun Lu

RNA biology and glycobiology were considered domains exclusive to each other until the discovery of glycoRNA in 2021. In recent studies, RNAs, including glycoRNAs, has been identified on mammalian cell surface, a topologically distinct space from the nucleus/cytoplasm, where the majority of cellular RNAs are traditionally found. Those findings raise numerous intriguing questions: What are the functions of cell surface RNA/glycoRNA? How are those RNAs transported and located on cell membrane? What is the chemical nature of glycoRNA... Addressing these questions will undoubtedly be of paramount importance for both the science and RNA society.

Since joining the Lu lab in 2021, I used neutrophil to study the cell surface RNAs/glycoRNAs. My recent study revealed the critical role of cell surface RNAs in neutrophil recruitment to inflammatory sites. Neutrophils contain glycoRNAs, primarily small RNAs mapping to noncoding transcripts. These glycoRNAs, predominantly located on the cell surface, play a vital role in governing the initial capture and rolling of neutrophils on blood vessel walls through the recognition by endothelial P-seletin. I also found that glycoRNAs are surprisingly stable, they are produced and transported to cell surface in a cell autonomous manner. Knockdown of murine *Sidt* RNA transporters genes, abolishes neutrophil glycoRNAs and functionally mimics the loss of cell surface RNAs. In summary, our study illustrates the biological importance of cell surface glycoRNAs, shedding light on the regulatory pathway governing glycoRNA production.

As the cell surface RNA/glycoRNA field is at nascent stage, many exciting questions require future explorations to address. My focus will center on several key aspects, including deciphering the code for RNA glycosylation, identifying specific types of glycoRNA that interact with P-selectin, understanding the chemical nature of glycoRNAs, developing methods for labeling and imaging cell surface RNA/glycoRNA in situ, investigating the mechanisms through which glycoRNA is anchored on the cell membrane (I have some evidence that glycoRNA is most likely protected by protein binding) and setting standard for glycoRNAs purification/sequencing. Given that glycoRNAs can be found in many cell types, which is corroborated by our unpublished observations, I speculate that glycoRNAs could play important functions across multiple cell types and in multiple biological settings. Studying cell surface RNA/glycoRNA will open new perspectives for understanding hidden aspects of RNA biology and contribute to RNA society.

How to Apply

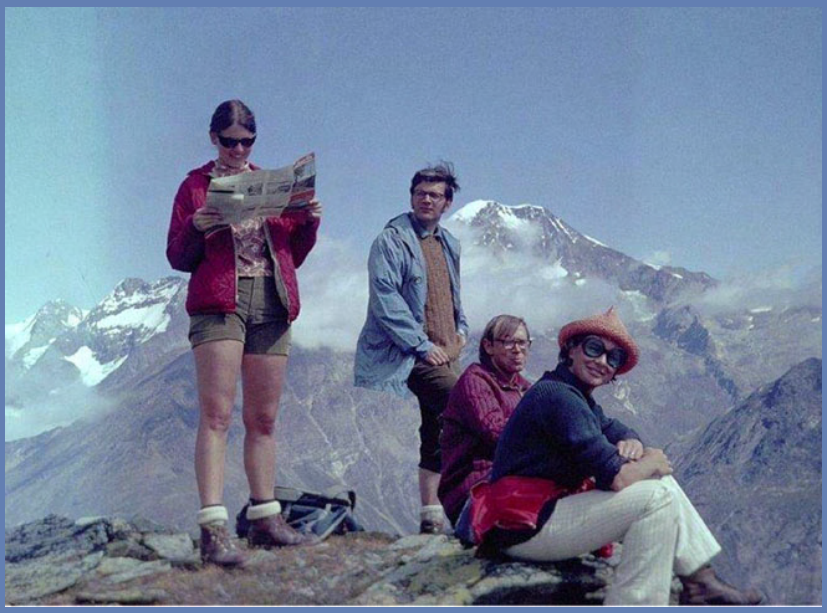
Eligibility

All current Yale undergraduate, Graduate and Post-doctoral Researchers are eligible.

Selection

An annual call for applications is made in January of each year. A committee of RNA Center Faculty members evaluates and selects awardees based on their personal essay, CV, and two letters of recommendation.

Please direct questions about the application to karla.neugebauer@yale.edu (not a member of the selection committee).



Yale Center for RNA Science and Medicine