RNA Society Outstanding Career Researcher Award

Ryan Fink

University of Penn, Perelman School of Medicine

RNA Society Number: 6013

Resume

Ryan Nicholas Fink
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Current Position

- August 2020 to current: Molecular Biologist developing projects at Perelman School of Medicine at University of Penn under Dr. Zissimos Mourelatos.

Education

Institution	Degree	Major	Date
• Monmouth University	Bachelor	Molecular Biology	2020
• Ocean County College	NA	Biology	2017
• Ocean County College	Certification	Medical Assistant EKG Tech Phlebotomy	2015

Research and Professional Experience	Position	Dates
• RNA Biologist University of Penn	Entry Level Researcher	2020-Present
• Hicks Lab, Department of Biology, Monmouth University	Undergraduate Research	2018-2020
• Robert W Johnson Barnabas Health	Medical Assistant	2017-2018
• Southern Ocean Pediatrics	Medical Assistant	2015-2017

First Author Publications

- Accepted to *Nucleic Acids Research*: Pre-mRNA Structure Probing Uncovers Therapeutic Targets: SHAPE-MaP Analysis of Telescripting and Cryptic Polyadenylation Signal Motifs

Honors and Awards

- Academic Achievement Award for Highest GPA in Molecular Cell Physiology Major Class of 2020
- Biology Research Achievement Award, Monmouth University, 2020
- American Society for Gene and Cell Therapy Poster Presenter, 2020
- American Society for Biochemistry and Molecular Biology Honor Society Chi Omega Lambda, 2020
- RNA Symposium Travel Grant and Presenter, 2020
- American Society for Biochemistry and Molecular Biology Presenter and Travel Grant, 2020
- American Society for Biochemistry and Molecular Biology Undergraduate Research Award, 2019
- RNA Institute Symposium Presenter, 2019
- Dean's List, Monmouth University, 2018-2020
- Senior Mentorship Program Mentor for High Technology Highschool, 2019

- Monmouth University Summer Research Grant, 2018, 2019
- Schering-Plough Undergraduate Research Scholarship, 2018
- UMBC Undergraduate Research Symposium Presenter, 2018
- Volunteer, Ocean County Jail, 2017-2018

Professional Membership	**Dates**
• American Society for Gene and Cell Therapy	2018-Current
• New York Academy of Sciences	2018-Current
• American Society for Biochemistry and Molecular Biology	2018-current

Clubs	**Dates**
• Event Coordinator at Next-Gen Science Club	2018-Current

Letter of Application

I began my research career in the lab of Dr. Martin Hicks at Monmouth University. The Hick's lab is currently studying RNA splicing, structure, and RNA therapeutics focusing on a new treatment for Glioblastoma Multiforme (GBM). A prevalent mutation in GBM is the overexpression and constitutive activation of Epidermal Growth Factor Receptor (EGFR). EGFR is a membrane bound tyrosine kinase receptor with an extracellular domain that binds Epidermal Growth Factor (EGF). The wild-type EGFR mRNA has 28 exons, with 10-exon and 16-exon alternatively spliced isoforms found in nature. The 10-exon isoform is translated into the extracellular domain only and previous literature shows it is secreted sequestering EGF in the extracellular space, making it an ideal therapeutic to treat GBM. Briefly, we use an adeno-associated virus vector to deliver the therapy in *Mus musculus* and a GBM tissue culture model. The therapy vector has two main components. It has an 'antisense' region where we clone 20-40 nucleotides of a target transcript, which will bind complementary to a motif on a target RNA molecule, in addition to a G-quadruplex tail that recruits hnRNP H to aid in intron retention. The first project I began working on, I identified important splicing motifs within exon-10 and intron-10 of EGFR and cloned in the target sequences to the therapy vector. Specifically, I designed our therapy vector to target the 5' splice site (5'ss) at exon-10 of EGFR during splicing. Blocking the 5'ss kinetically favors the recognition of a downstream cryptic polyadenylation motif within intron 10 promoting intron retention and alternative polyadenylation, which yields the therapeutic isoform. I performed vector design, molecular cloning, tissue culture, RNA extraction, reverse transcription, and qPCR to assess a fold change in EGFR mRNA levels. I performed an ELISA assay using an antibody against wild-type EGFR. Additionally, I transfected HEK293t cells with a plasmid expressing hnRNP H, extracted and purified hnRNP H, and performed EMSA gels to prove that our therapy binds hnRNP H.

I wanted to research the role of RNA secondary structure and its relationship with alternative splicing outcomes. The intron retention and alternative polyadenylation mechanism assumes that the antisense RNA molecule is able to bind to the 5'ss undeterred by RNA secondary structure. I looked in the literature and found a method called SHAPE-MAP to study the structure of the indicated region. In vitro, I deduced a secondary structure for the exon-10, intron-10 region of EGFR. This included customizing buffers, RNA transcription and purification, RNA modification and folding, and library preparation. I sequenced in our lab on an Oxford Nanopore and interpreted the data myself using several command line programs. The important finding was that the structure of the cryptic polyadenylation motif is unavailable for binding. This led to my novel approach of designing an antisense RNA therapy that is aimed at unleashing a structurally hidden RNA sequence to alter splicing. I designed a therapy that targets an unstructured region directly upstream of the cryptic polyadenylation site; I transfected a GBM cell line, isolated RNA and performed qPCR. The results show that the therapy designed by structure induced a 7-fold increase of polyadenylation activation events is comparison to the therapy that targets the 5'ss. The results hopefully will be published by the end of 2021 pending review.

After two years of full-time work in the Hicks Lab, I joined the Mourelatos Lab at University of Penn. The Mourelatos lab has two main, current objectives. The first is to understand more about how piRNAs, a class of small RNAs that bind to PIWI proteins, are generated and their functions in the germ

line of two model systems, *Drosophila melanogaster* and *Mus musculus*. The second area involves ribothrypsis, a new pathway of cotranslational decay of canonical mRNAs mediated by endonucleolysis that his lab described.

When I joined the Mourelatos lab I assisted a senior research investigator with his piRNA focused projects and in the process, I became proficient in numerous methods: culturing and transfecting cells; protein and RNA immunoprecipitations; immunofluorescence and confocal microscopy; quantitative Western blots; and dissection of *Drosophila* ovaries.

Confident with my research skills, I developed my own project trying to set up an *in vitro* translation system to study ribothrypsis and more general cotranslational mRNA decay. We explore the hypothesis that ribothrypsis is an ancient mechanism that may operate in bacteria. I am using highly purified E. coli ribosomes with the PURE system (containing recombinant translation initiation, elongation and release factors along with tRNAs, tRNA synthetases) to test decay of specific, 5'end radiolabeled short mRNAs that I generate with in vitro transcription. Our preliminary results are quite encouraging, and my goal is to identify the nucleolytic activity from this highly purified system.

I also collaborate with a computational postdoc to uncover mRNA features that associate with ribothrypsis (mRNA cleavages during cotranslational mRNA decay). We use Akron-seq (degradome-Seq) to sequence the 5'end of mRNA fragments that we isolate from polyA RNA from human 293 cells. However, it is difficult to know if the 5'end is generated by cleavage or by exonucleolysis. XRN1 is a cytoplasmic 5' to 3' exonuclease that mediates mRNA decay and can degrade mRNAs after decapping or after internal cleavages. To remove confounding effects of XRN1 I used an XRN1 knockout 293 cell line that my PI had obtained from Dr. Valkov at the NIH. I first confirmed the absence of XRN1 protein with Western blots and I also confirmed that XRN2, a nuclear exonuclease involved in transcriptional termination and nuclear RNA processing was not upregulated and did not relocate to the cytoplasm to compensate for the loss of XRN1. I then prepared very high-quality total RNA from XRN1 knockout cells and from wild-type cells after treatment with translational inhibitors that primarily arrest elongation. I performed all tissue culture, treatment, and RNA isolation for more than 60 libraries. I did all experiments as biological triplicates. The libraries have all been sequenced and we are analyzing the results now. I am extremely fortunate to be in a lab culture where I am allowed to try so many new things and really be encouraged to think outside the box. My goals I set when I joined this lab were to learn as many new wet lab techniques as I could and get experience at a research intense institution.