BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Daniel Dominguez	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) DANIEL_DOMINGUEZ	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Texas at El Paso, El Paso, TX	BS	2007	Mathematics and Biology
University of North Carolina Chapel Hill, Chapel Hill, NC	Ph.D.	2008-2014	Pharmacology
MIT, Cambridge, MA	Post-doc	01/2015-08/2018	Biology

A. Personal Statement

I am an Early Stage Principal Investigator and Assistant Professor of Pharmacology at UNC. I have started developing a long-term basic research program at the intersection of RNA processing in normal and disease biology, with a focus on neurodegenerative disease and cancer. My lab utilizes biochemical, molecular, and computational strategies to investigate unconventional and/or understudied mechanisms of RNA-protein interactions. Our overarching goal is to understand how RNA-protein interactions regulate gene expression.

Early work in my career centered on connections between cell signaling and RNA processing in regulation of the cell cycle. Using high-throughput RNA sequencing, I discovered that periodic regulation of RNA processing, including alternative splicing and alternative polyadenylation, during the cell cycle was dependent on the activity of a kinase, CLK1 (Dominguez, et al. *eLife*, 2016). Building on those findings, I found that certain periodically expressed genes could serve as survival classifiers in cancer across multiple tumor types (Dominguez, et al. *Cell Research*, 2016). Using computational approaches, I helped discover RNA processing defects in a range of tumor types (Tsai, et al., *Oncotarget*, 2015), which, at the time, was a largely unexplored area using genomics techniques.

Later, as a postdoc, I explored the basic biochemical mechanisms driving RNA interactions with RBPs. While many classes of RBPs were first described over three decades ago, the repertoire of RNA sequences and cellular targets that these proteins bind is still largely unknown. I developed a high-throughput version of an assay (RNA bind-n-seq) that could determine the RNA specificity of a large number of RBPs. I profiled ~80 RBPs and discovered unappreciated mechanisms of RNA-protein interactions. My findings present the largest in vitro characterization of human RBPs. In this thorough study I demonstrated that the specificity of RBPs goes beyond short sequences (3-8 nucleotides in length), with most proteins having varied preferences for RNA secondary structure, flanking sequence context or dis-contiguous spaced motifs, thus changing the way we think about RNA-RBP interactions (Dominguez et al., *Molecular Cell*, 2018; Van Nostrand et al. *Nature*, 2019). Additionally, I took a cell-based approach to understand the role of RBPs in drug response in cancer, as drug resistance is a major blockade in the development of treatments. I successfully applied for an NRSA F32 fellowship from the NCI and have carried out a systematic analysis of how RBPs modulate drug sensitivity in cancer cells. As a postdoc I also oversaw research activities in the Burge lab of a large-multicenter grant supported by the Encyclopedia of DNA Elements (ENCODE).

Now, in my independent lab, I use various high-throughput binding assays combined with in-cell approaches to i) study the RNA specificity of unconventional RNA binding domains made up of low-complexity amino acid sequences; ii) determine how disease-associated RBPs (that are frequently mutated) differentially interact with cellular targets. The proposed research combines my expertise from all stages of my career thus far and allow me to test questions that I am uniquely qualified to study and that differ from that of my previous mentors. Overall, with this approach, I expect to be able to explore answers to important questions such as i) how is the cellular transcriptome regulated? ii) What are basic mechanisms of RNA-protein interactions? iii) How do specific interactions between RNAs and proteins regulate cellular processes in normal and pathogenic settings?

Critical to reaching these research goals are the work of my present and future lab members. I have established my lab which includes undergraduates (2), graduate students (4) and a research technician (1). My students both have successfully attained funding. I am deeply committed to mentorship and have hosted summer undergraduates and even high-school students for research experiences. I serve(d) on 7 thesis committees and have taught a core graduate level pharmacology course lecture on RNA therapeutics. Finally, I have a strong commitment to training and mentoring students and postdocs from diverse backgrounds for the betterment and advancement of the scientific enterprise.

Relevant publications are below:

a. **Daniel Dominguez*§**, Peter Freese*, Maria S Alexis*, Amanda Su, Myles Hochman, Tsultrim Palden, Cassandra Bazile, Nicole J Lambert, Eric L Van Nostrand, Gabriel A Pratt, Gene W Yeo, Brenton Graveley, and Christopher B Burge§. "Sequence, Structure and Context Preferences of Human RNA Binding Proteins." ***Molecular Cell*** 70, no. 2, (2018): 854-864. PMCID: PMC6062212. §***co-corresponding***

b. Eric L Van Nostrand, Peter Freese, Gabriel A Pratt, Xiaofeng Wang, Xintao Wei, Steven M Blue, **Daniel Dominguez**, Neal A L Cody, Sara Olson, Balaji Sundararaman, Rui Xiao, Lijun Zhan, Cassandra Bazile, Louis Philip Benoit Bouvrette, Jiayu Chen, Michael O Duff, Keri Garcia, Chelsea Gelboin-Burkhart, Abigail Hochman, Nicole J Lambert, Hairi Li, Thai B Nguyen, Tsultrim Palden, Ines Rabano, Shashank Sathe, Rebecca Stanton, Ashley L Louie, Stefan Aigner, Julie Bergalet, Bing Zhou, Amanda Su, Ruth Wang, Brian A Yee, Xiang-Dong Fu, Eric Lecuyer, Christopher B Burge, Brenton Graveley, Gene W Yeo. "A Large-Scale Binding and Functional Map of Human RNA Binding Proteins." ***Nature*** (*in press*)

c. Raeann Goering, Laura I. Hudish, Bryan B. Guzman, Nisha Raj, Gary J. Bassel, Holger A. Russ, **Daniel Dominguez**, J. Matthew Taliaferro. "FMRP promotes RNA localization to neuronal projections through interactions between its RGG domain and G-quadruplex RNA sequences." ***bioRxiv***, 2019

B. Positions and Honors

Positions

01/2015 - 08/2018 Postdoctoral Fellow, Chris Burge Lab, Biology, MIT
09/2018 - present Assistant Professor, Dept. of Pharmacology, Lineberger Comprehensive Cancer Center, and Bioinformatics and Computational Biology, UNC Chapel Hill

Academic and Professional Honors

2008 Director's Award, University of North Carolina at Chapel Hill
2008 State of North Carolina Graduate School Science and Technology Fellowship Director's Award
2008 Initiative to Maximize Student Development NIH Scholar Grant UNC Chapel Hill
2010 Department of Pharmacology Training Grant UNC Chapel Hill
2010 Student Collaborative Research Project Award UNC Chapel Hill
2013 Selected Speaker, CSHL Eukaryotic mRNA Processing Meeting, Cold Spring Harbor, NY
2014 Gordon Research Seminar Award, Newport, RI
2016 National Cancer Institute, F32 Ruth Kristen Postdoctoral Research Fellowship
2017 Invited Speaker, H3 Biomedicine, Cambridge, MA
2017 Invited Speaker, Novartis, Cambridge, MA
2017 Burroughs Wellcome Fund Postdoctoral Enrichment Fellowship
2017 Invited Speaker, Max Planck-Chinese Academy of Sciences Otto Warburg Research Symposium
2017 Invited Lecturer, Max Planck-Chinese Academy of Sciences Otto Warburg Summer School
2017 Selected Speaker, CSHL Eukaryotic mRNA Processing Meeting, Cold Spring Harbor, NY
2018 Chair of 2018 Gordon Research Seminar, Sunday River, ME
2018 Selected Speaker, GRC, Post-Transcriptional Gene Regulation, Sunday River, ME
2019 Invited Speaker, Symposium on RNA Biology, NC RNA Society, Durham, NC

C. Contributions to Science

1. Characterization of RNA binding protein specificity: To determine the RNA binding preference of a comprehensive set of RBPs I established a high-throughput version of the RNA bind-n-seq assay—a robust biochemical method to determine the sequence, structure and context preference of human RBPs in an unbiased fashion. This assay works by incubating a recombinant RBP with billions of unique RNA molecules. Subsequently, bound RNA molecules are isolated and prepared for RNA sequencing. Enriched RNA sequences and structures can then be determined by comparing protein-bound RNAs to input RNAs. As the production, maturation, localization, modification, translation, and degradation of cellular RNAs is primarily controlled by RBPs, determining how RBPs bind RNA is essential for a more complete understanding of both normal and disease-associated RNA processing. I profiled the affinity landscape of 78 human RNA binding proteins and demonstrated the importance of RBP specificities in regulation of RNA processing through integrative analysis with *in vivo* binding and loss of function studies. I found that many RBPs bind similar short sequence elements, but specificities across factors diverge when additional features such as secondary structure, bipartite (or split dis-contiguous) motifs and nucleotide context are considered. In total I have authorship in 2 additional manuscripts in late stages of review and 2 corresponding author manuscripts under preparation, all dealing with RBP-RNA biology or RNA processing.

a. **Daniel Dominguez*§**, Peter Freese*, Maria S Alexis*, Amanda Su, Myles Hochman, Tsultrim Palden, Cassandra Bazile, Nicole J Lambert, Eric L Van Nostrand, Gabriel A Pratt, Gene W Yeo, Brenton Graveley, and Christopher B Burge§. "Sequence, Structure and Context Preferences of Human RNA Binding Proteins." ***Molecular Cell*** 70, no. 2, (2018): 854-864. PMCID: PMC6062212. §***co-corresponding***

b. Eric L Van Nostrand, Peter Freese, Gabriel A Pratt, Xiaofeng Wang, Xintao Wei, Steven M Blue, **Daniel Dominguez**, Neal A L Cody, Sara Olson, Balaji Sundararaman, Rui Xiao, Lijun Zhan, Cassandra Bazile, Louis Philip Benoit Bouvrette, Jiayu Chen, Michael O Duff, Keri Garcia, Chelsea Gelboin-Burkhart, Abigail Hochman, Nicole J Lambert, Hairi Li, Thai B Nguyen, Tsultrim Palden, Ines Rabano, Shashank Sathe, Rebecca Stanton, Ashley L Louie, Stefan Aigner, Julie Bergalet, Bing Zhou, Amanda Su, Ruth Wang, Brian A Yee, Xiang-Dong Fu, Eric Lecuyer, Christopher B Burge, Brenton Graveley, Gene W Yeo. "A Large-Scale Binding and Functional Map of Human RNA Binding Proteins." ***Nature*** (*in press*)

2. RNA Processing in cell cycle control and cancer: To understand gene expression dynamics during the cell cycle I used RNA sequencing to profile the transcriptome of synchronously dividing cells. I identified >1,000 genes whose expression levels were periodic and demonstrated that long non-coding RNAs are also periodically expressed. Through an integrative analysis of periodic gene expression and hundreds of transcription factor and histone modifications, and Chromatin Immunoprecipitation (ChIP) assays, I identified chromatin-binding proteins (e.g. CTCF) and histone modifications (e.g. H3K36me3) associated with periodic gene expression. Using thousands of tumor gene expression data sets from The Cancer Genome Atlas (TCGA), I showed that tumors can be classified based on their expression of cell cycle or periodic genes. Importantly, these classifiers predict survival across a broad range of tumors derived from various tissues and tumor subtypes.

Despite well-known cell-cycle-dependent gene expression changes or periodic gene expression, alternative splicing of RNAs in a periodic manner had never been demonstrated. Subsequently, I identified ~1,300 genes with cell cycle-dependent alternative splicing changes. Many of these genes were significantly enriched in functions linked to cell cycle control, DNA damage response and cancer-related pathways. I also identified a protein kinase, CLK1, whose level was also under cell cycle-dependent fluctuations via an auto-inhibitory circuit and whose activity controlled periodic splicing. Disruption of CLK1 activity led to pleiotropic cell cycle defects and loss of proliferation, whereas over-expression of CLK1 was associated with various cancers. Importantly, this work was the first example of this RNA processing step being controlled in a cell cycle-dependent manner and has profound implications for the way the field views gene expression dynamics during cell division. Overall, my work demonstrated an intimate connection between splicing, cell division and cancer.

Additional studies explored alternative splicing landscapes in tumor cells. In one computational project, we identified thousands of splice isoforms that are altered in breast, lung and kidney cancer. In another study we demonstrated that a specific splicing factor; RBM4, acts as a tumor suppressor and antagonizes the activity of SRSF1, an oncogenic splicing factor. Finally, although not yet published, I recently identified a subset of renal cancers (clear cell renal cell carcinoma) that express high levels of transcripts with retained introns. Strikingly, patients with these tumors have significantly decreased overall survival, compared to patients with tumors that

have normal splicing patterns. I have linked the MTOR signaling pathway to these alterations in intron retention and use much of this study as preliminary data.

a) **Daniel Dominguez,** Yi-Hsuan Tsai, Robert Weatheritt, Yang Wang, Benjamin J. Blencowe, and Zefeng Wang. "An extensive program of periodic alternative splicing linked to cell cycle progression." ***Elife*** 5 (2016): e10288. PMCID: PMC4884079

b) **Daniel Dominguez**, Yi-Hsuan Tsai, Nicholas Gomez, Deepak Kumar Jha, Ian Davis, and Zefeng Wang. "A high-resolution transcriptome map of cell cycle reveals novel connections between periodic genes and cancer." ***Cell Research*** 26, no. 8 (2016): 946-962. PMCID: PMC4973334

c) Yihsuan S. Tsai, **Daniel Dominguez**, Shawn M. Gomez, and Zefeng Wang. "Transcriptome-wide identification and study of cancer-specific splicing events across multiple tumors." ***Oncotarget*** 6, no. 9 (2015): 6825. PMCID: PMC4466652

d) Yang Wang, Dan Chen, Haili Qian, Yihsuan S. Tsai, Shujuan Shao, Quentin Liu, **Daniel Dominguez**, and Zefeng Wang. "The splicing factor RBM4 controls apoptosis, proliferation, and migration to suppress tumor progression." ***Cancer Cell*** 26, no. 3 (2014): 374-389. PMCID: PMC4159621

Complete list of my published works at:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1PSU3Yv6QBpk1/bibliograpahy/49993334/public/?sort=date&direction=ascending