

BIOGRAPHICAL SKETCH

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NAME: Joseph Mauro Calabrese

eRA COMMONS USER NAME (credential, e.g., agency login): jmcablr

POSITION TITLE: Assistant Professor of Pharmacology

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

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---------------------------|----------------------------|--------------------------------|
| University of Wisconsin-Madison | B.S. | 12/01 | Chemistry and Biochemistry |
| Massachusetts Institute of Technology | Ph.D. | 02/08 | Molecular Biology and Genomics |
| University of North Carolina at Chapel Hill | Postdoctoral Fellowship | 02/14 | Genetics and Genomics |

A. Personal Statement

The long-term goals of my laboratory are to understand, at the molecular level, how non-protein coding RNA regulates the transcriptional output of mammalian genome and apply knowledge gained to understand the molecular events that give rise to and sustain human disease. I have been studying RNA-mediated gene regulation since 2002. In graduate school, as a post-doctoral fellow, and now as a PI, I have taken discovery-driven approaches to make vertical advances in knowledge that have impacted broad areas in biomedical science; collectively, my first and second author publications have been cited over 1,000 times since 2006. I joined the Department of Pharmacology and Lineberger Comprehensive Cancer Center in 2014 to establish a research program investigating mechanisms of long noncoding RNAs (lncRNAs) as they relate to epigenetic regulation in cancer and other human diseases. Current research in the lab focuses on understanding how intrinsic sequence elements within lncRNAs and the trans-acting factors that bind them cooperate to induce specific epigenetic states at targeted regions within mammalian genomes.

B. Positions and Honors

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| 1997 | Order Sons of Italy Scholarship |
| 1998 | Undergraduate Researcher, University of Wisconsin-Madison |
| 2000 | Elvehjem Scholarship for Excellence in Biochemistry |
| 2001 | Phi Beta Kappa |
| 2001 | University of Wisconsin-Madison, Comprehensive Honors |
| 2002 | Tutor, University of Wisconsin-Madison |
| 2002 | Graduate Student, Phillip Sharp lab, MIT |
| 2007 | RNA Society Meeting abstract-selected speaker |
| 2008 | Post-doctoral Fellow, Terry Magnuson lab, UNC Chapel Hill |
| 2008 | UNC Lineberger Comprehensive Cancer Center Postdoctoral Training Award |

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| 2008 | UNC UCRF Pilot Project Award |
| 2009 | American Cancer Society Post-doctoral fellowship |
| 2012 | Keystone Symposia on Noncoding RNA abstract-selected speaker |
| 2012 | Keystone Symposia on Noncoding RNA Scholarship recipient |
| 2013 | Keystone Symposia on Noncoding RNA abstract-selected speaker |
| 2013 | UNC Chapel Hill Postdoctoral Research Excellence Award |
| 2014 | Assistant Professor of Pharmacology, UNC Chapel Hill |
| 2015 | March of Dimes Basil O'Connor Scholar |

C. Contributions to Science (chronological)

For each contribution, indicate the historical background that frames the scientific problem; the central finding(s); the influence of the finding(s) on the progress of science or the application of those finding(s) to health or technology; and your specific role in the described work.

1) *Discovering functions of microRNAs in embryonic stem cells.*

I joined Phil Sharp's lab in 2003, shortly after the discovery of RNAi in mammals. At that time, little was known of the functions that micro- and other small RNAs played in most cell types, including in mouse embryonic stem cells. Nevertheless it was clear at the time that microRNAs played important roles in stem cell biology, human development, tissue regeneration, and cancer. As a graduate student, I derived mouse embryonic stem cells homozygous for a conditional allele of *Dicer*, a gene central to RNAi, and characterized the mutant phenotypes after knockout. This work led to several observations that have relevance to human health. Collaboration with post-doctoral fellow AK Leung led to one of the early publications linking microRNA function to cellular stress responses (Leung *PNAS* 2006). Using a conditional deletion strategy that I helped to develop, two graduate students discovered a role for the conserved microRNAs 290-295 in modulating apoptosis (Zheng, Ravi *PLoS* 2011). A large portion of my own thesis was spent developing experimental and computational methods to profile, via high-throughput sequencing, small RNA populations in *Dicer* wild-type and knockout cells. The most significant finding to result from this work in regards to RNAi was a quantitative cataloguing of the microRNAs expressed in embryonic stem cells. This work has had broad utility to biomedical research communities since its publication (Calabrese *PNAS* 2007); to date, it has been cited 218 times.

Leung, A.K., **Calabrese, J.M.**, and Sharp, P.A. 2006. Quantitative analysis of Argonaute protein reveals microRNA-dependent localization to stress granules. *Proc Natl Acad Sci U S A* 103(48): 18125-18130. PMC1838717

Calabrese, J.M.*, Seila, A.C.*, Yeo, G.W., and Sharp, P.A. 2007. RNA sequence analysis defines Dicer's role in mouse embryonic stem cells. *Proc Natl Acad Sci USA* 104(46): 18097-18102. PMC2084302

Zheng, G.X.*, Ravi, A.*, **Calabrese, J.M.**, Medeiros, L.A., Kirak, O., Dennis, L.M., Jaenisch, R., Burge, C.B., Sharp P.A.. 2011. A latent pro-survival function for the mir-290-295 cluster in mouse embryonic stem cells. *PLoS Genet.* 2011 May;7(5):e1002054. PMC3088722

2) *Discovering conserved principles of the transcriptional process through genomic approaches.*

The advent of next-generation sequencing in 2005 allowed small RNA populations in cells to be profiled at an unprecedented level. Phil Sharp was an early advocate and encouraged post-doctoral fellow AC Seila and myself to undertake a large study to sequence small RNA populations in wild-type and *Dicer* knockout cells. In addition to significant findings described above, Dr. Seila and I made the unexpected observation that small RNAs that were not *Dicer* dependent were produced in both the sense and anti-sense directions relative to transcription start sites of most expressed genes. Considered in context with ChIP-Seq data from Richard Young's lab, additional biochemical data produced by AC Seila, and two separate studies that were ultimately co-published with ours, these data indicated that most

mammalian promoters were capable of, and frequently engaged in, divergent initiation events (Seila, Calabrese *Science* 2008; others listed below). These works fundamentally altered our perception of the mammalian transcriptional process and have had profound implications for our understanding of gene regulation and evolution. Divergent transcription is thought to help maintain promoters in a state poised for regulation and is hypothesized to be a source of RNA that has led to the evolution of several, conserved lncRNAs.

Seila, A.C.*, **Calabrese, J.M.***, Levine, S.S., Yeo, G.W., Rahl, P.B., Young, R.A., and Sharp, P.A. 2008. Divergent transcription from active promoters. *Science* 322:1849-1851. PMC2692996

Core, L.J., Waterfall, J.J., Lis, J.T. 2008. Nascent RNA Sequencing Reveals Widespread Pausing and Divergent Initiation at Human Promoters. *Science*. 322: 1845-1848. PMC2833333

Preker, P., Nielsen, J., Kammler, S., Lykke-Andersen, S., Christensen, M.S., Mapendano, C.K., Schierup, M.H., Jensen, T.H. 2008. RNA Exosome Depletion Reveals Transcription Upstream of Active Human Promoters. *Science*. 322:1851-1854.

3) *Discovering principles of lncRNA/genome interactions.*

As I finished my thesis in 2007, work from many groups brought renewed attention to the roles that lncRNAs played in gene regulation. In 2008 I joined Terry Magnuson's lab as a post-doctoral fellow to study the molecular mechanisms of one of the most conserved lncRNAs known, *Xist*. *Xist* is the flagship example of what now appears to be a large class of lncRNAs, many of which are essential for human development and misregulated in cancer, that function to regulate gene expression *in cis*, on their chromosomal allele of synthesis. Despite its discovery more than 20 years ago, the mechanisms by which *Xist* interfaces with its genic targets on the inactive X chromosome and ultimately induces their silencing remain poorly defined. To begin to address this fundamental gap in knowledge, I took advantage of recent developments in the mouse genetics community and the continued improvement of high-throughput sequencing technology to develop methods to map the effect that *Xist* and similar monoallelically expressed lncRNAs exert on their local epigenetic environment. Through this work I made several unexpected observations, perhaps the most notable of which was that regulatory elements along the inactive X chromosome harbored a unique epigenetic signature suggestive of an active role in targeting *Xist* to the genome. At its culmination, this work was published in *Cell*. Evidence supporting our proposed notion that certain lncRNAs interpret regulatory elements along chromosomes to achieve their specified functions continues to accumulate, including our most recent observations which raise the possibility that mRNA produced from gene bodies helps target *Xist*-like lncRNAs to gene dense regions. In addition to the impact this work has had on the lncRNA and X-inactivation fields, the computational methods and experimental system I developed for allele-specific genomic analyses have played central roles in several studies, three of which are referenced below.

Calabrese, J.M., Sun, W., Song, L., Mugford, J.W., Williams, L., Yee, D., Starmer, J., Mieczkowski, P., Crawford, G.E., Magnuson, T. 2012. Site-specific silencing of regulatory elements as a mechanism of X-inactivation. *Cell* 151(5): 951-63. PMC3511858

King I.F., Yandava C.N., Mabb A.M., Hsiao J.S., Huang H.S., Pearson B.L., **Calabrese J.M.**, Starmer J., Parker J.S., Magnuson T., Chamberlain S.J., Philpot B.D., Zylka M.J. 2013. Topoisomerases facilitate transcription of long genes linked to autism. *Nature* 501(7465): 58-62. PMC3767287

Williams R.L.*, Starmer J.*, Mugford J.W., **Calabrese J.M.**, Mieczkowski P., Yee D., Magnuson T. 2014. A Method for Determining Chromosomal Interactions in 4C-Seq Data. *Nucleic Acids Research* 42(8): e68. PMID: PMC4005674.

Mugford J.W., Starmer J., Williams R.L., **Calabrese J.M.**, Mieczkowski P., Yee D., Magnuson T. 2014. Evidence for local regulatory control of escape from imprinted X chromosome inactivation. *Genetics*. 197(2):715-723. PMC4063926.

4) Defining the impact of genomic imprinting on transcriptional output in mammals.

Genomic imprinting is an epigenetic process initiated during mammalian gametogenesis, which results in preferential expression of genes from one parentally inherited allele over the other. Over one hundred fifty imprinted genes have been identified in mammals. As a class, these genes play important roles in development, growth, metabolism, and social adaptation. Defects in imprinting can cause cancer, in the form of Wilm's Tumor, and other human diseases, including Angelman, Prader-Willi, Beckwith-Wiedemann, and Silver-Russell Syndromes. Faithful maintenance of imprinting also plays an important role in reprogramming and maintenance of stem cell identity. However, in large part due to the technical difficulty of measuring transcriptional outputs of single alleles in diploid mammalian nuclei, the global effects of imprinting on transcription have been more difficult to define. Using methods for allele-specific transcriptome analysis that I developed during my postdoctoral fellowship, my lab, in collaboration with Terry Magnuson's lab at UNC, recently performed an unbiased profiling of imprinted gene expression in a mouse stem cell population where imprinting is known to play an essential role. Our analysis provides arguably the most quantitative benchmarking of the transcriptional effects that imprinting exerts within a single cell type to date, supports a newly posited theory for the origins and functions of the process in mammals (Radford 2011), suggests a central role for conserved lncRNAs in carrying out the functions of imprinting in cells, and establishes an experimental foundation upon which to dissect mechanisms that underpin imprinted gene expression in mammals.

Calabrese J.M.^{ca}, Starmer J., Schertzer M.D., Yee D., Magnuson T.^{ca}. 2015. A Survey of Imprinted Gene Expression in Mouse Trophoblast Stem Cells. G3. Epub ahead of print. PMC in process by Journal.

Radford EJ, Ferrón SR, Ferguson-Smith AC. 2011. Genomic imprinting as an adaptive model of developmental plasticity. *FEBS Lett.* 585(13):2059-66. PMID:21672541.

*s denote equal contribution.

^{ca} denotes co-corresponding authorship.

Complete list of Published work (15 peer-reviewed publications):

<http://www.ncbi.nlm.nih.gov/sites/myncbi/j>

mauro.calabrese.1/bibliography/44735528/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

Laboratory Start-up Funds (Calabrese)

3/1/2014 –

Lab Goals: Our long-term goals are to understand how non-protein coding RNA regulates the transcriptional output of mammalian genomes and apply knowledge gained to understand the molecular events that give rise to and sustain human disease.

JMC Role: Principal Investigator

March of Dimes Basil O'Connor Starter Scholar Award (Calabrese)

2/1/2015-1/31/2017

Selective modulation of noncoding RNA function as a tool to treat childhood disease

Project Goals: Define mechanisms by which the *Kcnq1ot1* long noncoding RNA represses expression of disease-associated genes within 11p15. Apply knowledge gained to develop new strategies to modulate its repressive activity in childhood disease.

JMC Role: Principal Investigator

UNC Faculty Development Award (Calabrese)

1/1/2015-12/31/2015

Defining mechanisms of interface between long noncoding RNAs and their genomic targets

Project Goals: Perform an RNAi screen to discover the RNA/DNA binding proteins that are essential for the *Xist* lncRNA to silence gene expression.

JMC Role: Principal Investigator

1 R01 GM105785-01A1 (Sun)

5/15/2014-4/30/2018

National Institute of General Medical Sciences

Statistical methods for RNA-Seq data analysis

Project Goals: Develop statistical methods and software for allele-specific RNA-seq data analysis in humans, to help biomedical researchers translate knowledge accumulated in DNA variations and RNA-seq data into strategies of personalized disease prevention and treatment.

JMC Role: Collaborator

Completed Research Support

117571-PF-09-124-01-DDC (Calabrese) 7/1/2009 – 6/30/2012

American Cancer Society Post-Doctoral Fellowship

Chromosome-wide analysis of X-inactivation in the mouse

Project Goals: Perform a high-resolution allele-specific gene expression and chromatin microenvironments across the inactive X chromosome, in order to better understand how the *Xist* lncRNA functions.

JMC Role: Principal Investigator