OMB No. 3145-0279 (Expiration Date 10/31/2026)

NIH BIOGRAPHICAL SKETCH COMMON FORM

Name: Calabrese, J Mauro

Persistent Identifier (PID) of the Senior/Key Person: https://orcid.org/0000-0002-1213-2540

Position Title: Associate Professor of Pharmacology

Organization and Location: University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

PROFESSIONAL PREPARATION

INSTITUTION AND LOCATION	DEGREE	Start Date	Completion Date	FIELD OF STUDY
University of North Carolina at Chapel Hill, Chapel Hill , NC, USA	Postdoctoral Fellow	03/2008	02/2014	Genetics and Genomics
Massachusetts Institute of Technology, Boston, MA, USA	DOCTOR OF PHILOSOPHY	09/2002	02/2008	Molecular Biology and Genomics
University of Wisconsin-Madison, Madison, WI, USA	BACHELOR OF SCIENCE	09/1997	12/2001	Chemistry and Biochemistry

Appointments and Positions

2020 - present	Associate Professor of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States
2022 - present	Co-Director, RNA Society of North Carolina, Chapel Hill, North Carolina, United States
2022 - present	Co-Director, RNA Discovery Center, Chapel Hill, North Carolina, United States
2020 - present	Director of Graduate Studies, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

Products

Products Closely Related to the Proposed Project

1. Calabrese JM, Sun W, Song L, Mugford JW, Williams L, Yee D, Starmer J, Mieczkowski P, Crawford GE, Magnuson T. Site-specific silencing of regulatory elements as a mechanism of X inactivation. Cell. 2012 Nov 21;151(5):951-63. PubMed Central PMCID: PMC3511858.
2. Schertzer MD, Braceros KCA, Starmer J, Cherney RE, Lee DM, Salazar G, Justice M, Bischoff SR, Cowley DO, Ariel P, Zylka MJ, Dowen JM, Magnuson T, Calabrese JM. lncRNA-Induced Spread of Polycomb Controlled by Genome Architecture, RNA Abundance, and CpG Island DNA. Mol Cell. 2019 Aug 8;75(3):523-537.e10. PubMed Central PMCID: PMC6688959.
3. Trotman JB, Lee DM, Cherney RE, Kim SO, Inoue K, Schertzer MD, Bischoff SR, Cowley DO, Calabrese JM. Elements at the 5' end of Xist harbor SPEN-independent transcriptional antiterminator activity. Nucleic Acids Res. 2020 Oct 9;48(18):10500-10517. PubMed Central PMCID: PMC7544216.
4. Trotman JB, Abrash EW, Murvin MM, Braceros AK, Li S, Boyson SP, Salcido RT, Cherney RE, Bischoff SR, Kaufmann K, Eberhard QE, Zhang Z, Cowley DO, Calabrese JM. Isogenic comparison of Airn and Xist reveals core principles of Polycomb recruitment by lncRNAs. Mol Cell. 2025 Mar 20;85(6):1117-1133.e14. PubMed Central PMCID: PMC11932450.
5. Trotman JB, Porrello A, Schactler SA, DeLeon LE, Eberhard QE, Boyson SP, Zhang Z, Lee DM, Kirik SE, Sultana N, Nguyen SN, Beltejar MG, Shinn MK, Ong SE, Gonzalez-Perez MP, Shaffer SA, Dominguez D, Shechner DM, Calabrese JM. Xist Repeat A coordinates an assembly of SR proteins to recruit SPEN and induce gene silencing. bioRxiv. 2025 May 26; PubMed Central PMCID: PMC12154583.

Other Significant Products Highlighting Contributions to Science

1. Trotman JB, Li S, Eberhard QE, Zhang Z, Calabrese JM. Protocol for evaluating RNA-protein associations in mammalian cells with RIP-seq and RIP-qPCR. STAR Protoc. 2026 Mar 20;7(1):104298. PubMed Central PMCID: PMC12796723.
2. Schertzer MD, Thulson E, Braceros KCA, Lee DM, Hinkle ER, Murphy RM, Kim SO, Vitucci ECM, Calabrese JM. A piggyBac-based toolkit for inducible genome editing in mammalian cells. RNA. 2019 Aug;25(8):1047-1058. PubMed Central PMCID: PMC6633203.

3. Cherney RE, Mills CA, Herring LE, Braceros AK, Calabrese JM. A monoclonal antibody raised against human EZH2 cross-reacts with the RNA-binding protein SAFB. Biol Open. 2023 Jun 15;12(6) PubMed Central PMCID: PMC10259849.
4. Weidmann CA, Mustoe AM, Jariwala PB, Calabrese JM, Weeks KM. Analysis of RNA-protein networks with RNP-MaP defines functional hubs on RNA. Nat Biotechnol. 2021 Mar;39(3):347-356. PubMed Central PMCID: PMC7956044.
5. Kirk JM, Kim SO, Inoue K, Smola MJ, Lee DM, Schertzer MD, Wooten JS, Baker AR, Sprague D, Collins DW, Horning CR, Wang S, Chen Q, Weeks KM, Mucha PJ, Calabrese JM. Functional classification of long non-coding RNAs by k-mer content. Nat Genet. 2018 Oct;50(10):1474-1482. PubMed Central PMCID: PMC6262761.

Certification:

I certify that the information provided is current, accurate, and complete. This includes, but is not limited to, information related to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. § 6605.

In accordance with Section 10632 of the CHIPS and Science Act of 2022 (42 U.S.C. § 19232), each individual identified as a senior/key person must certify that they are not a party to a malign foreign talent recruitment program.

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Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§287, 1001, 1031 and 31 U.S.C. §§3729-3733 and 3802.

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NIH BIOGRAPHICAL SKETCH SUPPLEMENT

Name: Calabrese, J Mauro

Persistent Identifier (PID) of the Senior/Key Person: https://orcid.org/0000-0002-1213-2540

Position Title: Associate Professor of Pharmacology

Organization and Location: University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

Personal Statement

The central objective of research in my laboratory is to determine how long noncoding RNAs (lncRNAs) regulate gene expression. I have been studying RNA-mediated gene regulation since 2003 and allele-resolved epigenetic regulation governed by lncRNAs since 2008. Our research sits at an interface between RNA and chromatin biology, where we draw on knowledge from both fields to make mechanistic discoveries of fundamental relevance to human health. Over the last decade, our research has uncovered new insights into RNA-mediated epigenetic silencing and developed computational and experimental tools that have the potential to accelerate progress in many areas of research. These advances provide a foundation for dissecting the molecular mechanisms of lncRNAs throughout eukaryotes and place us on the cusp of major discoveries. Most relevant to the present application are our ongoing efforts to understand the mechanisms through which the Xist lncRNA induces gene silencing. In a study published as a preprint and which is currently under peer review, we discovered that the core splicing factor SRSF1 is critical for the optimal recruitment of SPEN by Xist, linking Xist to what we believe to be an ancient mechanism of transcriptional control, aspects of which may be conserved even in plants. In separate studies of the X chromosome and autosomal imprinted regions, we demonstrated ways in which lncRNA abundance, RNA-binding proteins, and specific DNA regulatory elements each modulate the intensity of lncRNA-induced gene silencing. We also found that within larger lncRNA-silenced domains, genes that are resistant to lncRNA-induced silencing coincide with accessible DNA regulatory elements that we hypothesize underlie resistance. En route to these discoveries, we developed and applied new approaches to map, quantify, and manipulate lncRNA-protein and protein-DNA interactions. We are now building on this foundation to the investigate the intersections between SPEN- and NuRD-directed silencing in the heart, leveraging our own expertise in allele-resolved studies of epigenetic regulation, X-chromosome inactivation, and SPEN, together with the expertise of the Conlon lab in developmental biology, physiology, and in vivo genomics. We expect the findings from our research to open new areas of inquiry and inspire new strategies to control gene expression therapeutically.

Honors

2022	Biomedical Scholar, Yang Family
2015	Basil O'Conor Scholar, March of Dimes
2013	Postdoctoral Research Excellence Award, UNC Chapel Hill
2009	Postdoctoral Fellowship, American Cancer Society

Contributions to Science

1. **Mechanisms of gene silencing induced by lncRNAs:**

Xist initiates silencing upstream of known histone-modifying enzymes, using mechanisms that remain unclear. Initiation requires Repeat A, a conserved tandem-repeat near Xist's 5' end, and SPEN, an ancient RBP that binds Repeat A and is recurrently inactivated in cancers. Paradoxically, both Repeat A and SPEN are also required for Xist production but the reasons why are unclear. In 2015, we worked with Howard Chang's laboratory to develop and apply ChIRP-MS to identify proteins that associate with Xist in vivo and implicate SPEN as critical for Xist-induced silencing. In the years since, we discovered that as a standalone transgene, a 2kb fragment of Xist that contains Repeat A and binds SPEN was incapable of inducing gene silencing. However, in that same transgenic context, Repeat A functioned as a transcriptional antiterminator that enabled read-through of downstream polyadenylation signal, suggesting an interaction with the transcriptional machinery that we believe is central to Repeat A's silencing and activating functions. In a follow-up study under review, we discovered the mechanistic basis for Repeat A's ability to recruit SPEN, bringing to light what may be a previously undescribed pathway of gene repression, whereby abnormal accumulations of SR-rich splicing factors target SPEN to nascent RNAs to induce their degradation and transcriptional repression. As part of these investigations, we developed a protocol to measure RNA-protein interactions that facilitates quantitative cross-sample comparisons, and exhibits increased dynamic range, similar post-lysis reassociation ratios, and a simplified workflow relative to CLIP/CLAP. See [Products Closely Related to the Proposed Project] and Other Sig Products #1.

2. **Mechanisms of interface between lncRNAs and their genomic targets:**

   The intensity of silencing induced by lncRNAs varies across genomic space. Some genes are susceptible to silencing by lncRNAs while others resist it. The mechanisms responsible are unknown. For the last 18 years, I have been using quantitative genomics to understand the mechanisms that underlie variation in silencing by lncRNAs. Our early work revealed an unexpected contour of silencing induced by Xist, in which genes that were subject to strong levels of silencing harbored a distinct chromatin signature but were often located in proximity to genes that escaped silencing, supporting the notion that expression of individual genes can be manipulated separately from the otherwise inactive X. More recently, we discovered that local intensity of lncRNA-induced repression can be modulated by DNA elements that interact through 3D space to control proximity to the lncRNA. In the same stem cell model, we found that genes that escape silencing are marked by CpG island promoters, high overall transcription, and avoidance of lncRNA contact. En route to these discoveries, we developed vectors to facilitate inducible genome editing by CRISPR, which have been distributed to >125 labs. We now hypothesize that escape from silencing is driven by the combined action of specific transcription factors, interacting proteins, and the act of transcription itself, which together enable individual genes to resist forming 3D contacts with proteinaceous condensates that surround repressive lncRNAs. Our work may identify approaches to potentiate lncRNA-induced silencing and drive escape from it, potentially inspiring approaches to treat congenital heart disorders, cancers, and forms of autoimmunity (e.g. lupus), where altering expression of lncRNA target genes is expected to be therapeutic. See [Products Closely Related to the Proposed Project] and Other Sig Products #2.

3. **Recruitment of Polycomb Repressive Complexes by lncRNAs:**

   Polycomb Repressive Complexes (PRCs) are essential enzymes and therapeutic targets in developmental disorders and cancers. PRCs are remarkably responsive to specific lncRNAs, for example, being recruited to chromatin within minutes after the lncRNA Xist is expressed. However, PRCs bind RNA with little sequence specificity and are inhibited by direct RNA binding, and how lncRNAs recruit PRCs to chromatin remains unclear. To gain insight, we began studying the lncRNAs Airn and Kcnq1ot1, which can each repress large (>3Mb) genomic regions on autosomes. We found that, like Xist, Airn and Kcnq1ot1 require the RNA-binding protein HNRNPK to recruit PRCs to chromatin. We demonstrated that Airn's repressive potency rivals that of Xist and scales with RNA abundance. We demonstrated that Airn's association with chromatin correlates in lockstep with local PRC recruitment. We discovered that a broadly-used monoclonal antibody raised against EZH2 (PRC2 catalytic subunit; >800 citations) cross-reacts with an RNA-binding protein called SAFB, underscoring the importance of orthogonal assays when studying interactions between chromatin-modifying enzymes and RNA. Our data support a bridged model for PRC recruitment, whereby lncRNAs interact with dedicated RNA-binding proteins that themselves bind and recruit PRCs. Similarly bridged interactions may underlie recruitment of other epigenetic regulatory enzymes by RNA. Our data likewise suggest that pathways that modulate the abundance of RNA on chromatin (splicing, polyadenylation, RNA turnover) may indirectly modulate intensity of RNA-mediated epigenetic control. Our work under this theme is determining how lncRNAs, RBPs, and epigenetic regulators interact to regulate transcription, establishing paradigms relevant to many aspects of gene regulation. See [Products Closely Related to the Proposed Project] and Other Sig Product #3.

4. **Relationships between sequence, structure, and function in lncRNAs:**

How RNA structures specify function in lncRNAs remains unclear. This is true even for Xist, the second lncRNA ever to be identified. In 2013, we collaborated with Dr. Kevin Weeks to apply technology developed in his lab, SHAPE-MaP, to identify structural properties of Xist ex vivo (on purified RNA) and in live cells. We optimized methods to detect in-cell RNA-protein interactions by SHAPE and discovered structural landmarks within Xist that appeared to be evolutionarily constrained. In follow-up work, we applied technology again developed in the Weeks lab called RNP-MaP to map, at nucleotide resolution, the regions within mouse and human Xist that interact with proteins in vivo. We found that the domains in Xist that are the most critical for silencing are the ones that interact the most robustly with proteins. This discovery provides a roadmap for structure-function studies in Xist and established RNP-MaP as a straightforward approach to identify functional domains in any lncRNA. Our work thus far has uncovered a remarkable layering of functional elements within Xist and supports an additive model, whereby the most critical structures in Xist (along with two other repressive lncRNAs called Airn and Kcnq1ot1) form over short distances to coordinate local protein interactions that in aggregate enact repression. Some of these structures may become targets of future drug-discovery campaigns. Our work aims to establish methods and analytical paradigms that enable studies of structure-function relationships in essentially any lncRNA. See Other Sig Product #4 and references therein.

5. **Methods to detect non-linear sequence similarity:**

   Progress in the lncRNA field has been stifled by a poor understanding of relationships between lncRNA sequence and function. LncRNAs are not constrained by codon usage, evolve rapidly, and achieve function by employing structures or proteins in ways that are not well-understood. Thus, lncRNAs with similar functions often lack linear sequence similarity. Yet, owing to a lack of alternatives, linear alignment remains central in lncRNA sequence comparison. As a result, studies of one lncRNA rarely inform the understanding of others, and among the thousands of unstudied lncRNAs, it remains challenging to identify those that harbor meaningful functions. We developed a concise approach, SEEKR (sequence evaluation through k-mer representation), that enables researchers to quantify sequence similarity via sequence substrings called k-mers. We used SEEKR to demonstrate that functionally analogous lncRNAs can harbor similarities that are invisible by conventional forms of linear alignment and to rationally design synthetic lncRNAs with pre-specified functions. Using SEEKR, we found that Xist and its presumed functional analogue in marsupials, the lncRNA Rsx, are each enriched in similar motifs despite lacking homology, highlighting a remarkable example of convergent evolution and suggesting a general model for how lncRNAs encode repressive function. We are developing derivatives of SEEKR that will be readily useable to novices and experts alike. When complete, these approaches will have transformative impacts, enabling investigators with little computational expertise to identify meaningful non-linear similarities between any nucleic acid species, including but not limited to lncRNAs. See Other Sig Product #5.

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