BIOGRAPHICAL SKETCH

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NAME: Jessica Ann Brown

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POSITION TITLE: Associate Professor of Biochemistry

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Wright State University (Dayton, OH)	B.S.	09/2000	03/2005	Chemistry & Biology
The Ohio State University (Columbus, OH)	Ph.D.	06/2005	12/2010	Biochemistry
Yale University (New Haven, CT)	Postdoc	02/2011	07/2016	Molecular & Structural Biology; Biochemistry

A. Personal Statement

My laboratory uses biochemical, structural and cellular approaches to discover and to understand the roles of RNA triple helices and their protein-binding partners in human health and disease. We are currently focused on the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) RNA triple helix, which protects the long noncoding RNA MALAT1 from degradation in cancer cells. We discovered that the MALAT1 triple helix is specifically recognized by methyltransferase-like protein 16 (METTL16), an N^6-methyladenosine (m^6A) RNA methyltransferase and the first putative triple-stranded RNA-binding protein. Thus, the short-term goals of my laboratory include structural characterization of the METTL16-MALAT1 triple helix complex and elucidation of METTL16's cellular function as a methyltransferase. In addition, we are trying to understand the fundamental structural and biochemical properties of triple helices, including interactions with small molecules. This research holds the potential to establish an entirely new class of RNA-binding proteins, to generate novel biomolecular tools to globally identify RNA triple helices (i.e. a triplexome) and to fill a gap of our basic understanding of triple helices. A long-term goal is to understand the secondary and tertiary structure of full-length human MALAT1, both in normal cells and in cancer cells.

Thus, my NIH-funded research requires structural biology tools. Although X-ray crystallography has been successful for the MALAT1 triple helix, solving structures of increasing complexity, such as the MALAT1 triple helix in complex with METTL16 or small molecules, requires "starting from scratch" and has not worked using a simple "soak-in" method. Therefore, my laboratory has explored cryo-EM because it can solve 3D structures of small RNAs, including small ligand-bound riboswitches, at resolutions of 3.5 Å or better. To date, my laboratory has been able to obtain only low-resolution structures (~5-8 Å) of the MALAT1 triple helix using cryo-EM. Because my laboratory is largely self taught, I feel that we could benefit greatly from formal training in an interactive, one-on-one setting. Therefore, I am applying to participate in NCCAT's TP1 Embedded Cross-Training Program during my sabbatical, which will begin late February 2024. Starting NCCAT training near the beginning of my sabbatical would allow me to use my knowledge gained from training to solve 3D structures at higher resolution.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022 – Present	Associate Professor, Department of Chemistry & Biochemistry, University of Notre Dame
2019 – Present	Member, American Society for Biochemistry & Molecular Biology
2017 – Present	Member, RNA Society
2016 – 2022	Assistant Professor, Department of Chemistry & Biochemistry, University of Notre Dame
2011 – 2016	Postdoctoral Fellow/Associate, Department of Molecular Biophysics & Biochemistry, Yale University, Supervisor: Prof. Joan A. Steitz
2009 – Present	Member, Philanthropic Educational Organization (P.E.O.)
2007 – Present	Member, American Association for the Advancement of Science (AAAS)
2005 – 2010	Graduate Fellow, Ohio State Biochemistry Program, The Ohio State University, Supervisor: Prof. Zucai Suo
2004 – 2005	Undergraduate Researcher, Department of Biochemistry and Molecular Biology, Wright State University, Supervisor: Prof. Steven J. Berberich

Honors

2023	Speaker, Gordon Research Conference on High Throughput Chemistry and Chemical Biology (New London, NH)
2023	Nominee, Rev. Edmund P. Joyce, C.S.C., Award for Excellence in Undergraduate Teaching
2017	Kavli Fellow, US National Academy of Sciences, Japanese-American-German Frontiers of Science Symposium (Bad Neuenahr, Germany)
2017	Speaker, Gordon Research Conference on Nucleic Acids (Biddeford, ME)
2016	Speaker, 18th Annual Rustbelt RNA Meeting (Cleveland, OH)
2016-2023	Clare Boothe Luce Assistant Professorship
2014-2019	NIH Pathway to Independence Award (K99/R00 GM111430)
2014	Aspen Cancer Conference Fellow and Carl Alden Scholar for Scientific Research Excellence
2012-2014	American Cancer Society Postdoctoral Fellowship (Grant 122267-PF-12-077-01-RMC)
2010	Ohio State University Edward J. Ray Travel Award for Scholarship and Service
2010	Ohio State University Presidential Fellowship
2008-2010	American Heart Association Predoctoral Fellowship (Grant 0815382D)
2008-2009	International P.E.O. Scholar Award for Women
2008 & 2009	Burrell Memorial Fund (Travel Award from Department of Biochemistry at Ohio State)
2008	Outstanding Oral Student Presentation Award for Molecular Life Sciences Interdisciplinary Graduate Programs Symposium at Ohio State
2007	Member, Phi Kappa Phi Honor Society
2005-2007	NIH Chemistry-Biology Interface Program Predoctoral Fellowship (Grant 5 T32 GM008512)
2005	Graduated *Summa Cum Laude*
2005	Wright State University Departmental Honors Scholar in Biological Sciences
2004-2005	Wright State University Fred White Scholarship
2002-2005	Wright State University College of Science and Mathematics Scholarship
2002	Member, National Society of Collegiate Scholars
2002	Member, Alpha Lambda Delta National Honor Society
2000-2005	Wright State University Valedictorian Scholarship
2000-2005	Dean's List

C. Contributions to Science

Structure and function of the MALAT1 RNA triple helix

Background: A triple-helical RNA stability element was found in polyadenylated nuclear (PAN) RNA, a long noncoding RNA produced by the Kaposi's sarcoma-associated herpesvirus (KSHV) during the lytic phase of infection. A similar structure was predicted to stabilize two abundant human long noncoding RNAs: MALAT1 and MENβ.

Findings: A similar, yet distinct, triple-helical element was established to be critical for the accumulation of both MALAT1 and MENβ in cells. Our crystal structure showed that the 3′ end of MALAT1 forms a bipartite

triple helix composed of nine U•A-U triples, a single C•G-C triple that is likely protonated *in vivo*, a C-G doublet, and two A-minor triples. The unique C^+•G-C triplet/C-G doublet functions to align the A-rich tract and U-rich loop to position the 3′-terminal A in a U•A-U triple, creating a blunt-ended triple helix that inhibits rapid nuclear RNA decay. Additionally, we discovered that the MALAT1 triple helix is specifically recognized by METTL16, an m^6A RNA methyltransferase, both in a test tube and in cell culture. Our biochemical results and a low-resolution 3D model of METTL16 bound to the MALAT1 triple helix indicate that the C-terminal domain of METTL16 interacts with the triple helix, particularly the CG doublet, whereas the N-terminal methyltransferase domain of METTL16 recognizes the basal linker-stem adjacent to triple helix.

Impact: As stability elements, the triple helices from MALAT1 and MENβ can be used as a tool to confer stability to inherently unstable cellular RNAs. This strategy is being implemented by other laboratory groups, for which we have provided plasmids for their studies. Importantly, the MALAT1 triple helix represents one of only three structurally validated RNA triple helices in eukaryotic cells. This discovery provides a special opportunity to learn more about the biology of RNA triple helices in cells. METTL16 binding to the MALAT1 triple helix has several important implications: RNA triple helices are structures that form inside cells and a class of triple-stranded RNA binding proteins likely exists as well as other RNA triple helices. This knowledge drives our current efforts to develop experimental tools that will enable the global discovery of naturally occurring RNA triple helices.

References:

a. **Brown, J.A.**, Valenstein, M.L., Yario, T.A., Tycowski, K.T. & Steitz, J.A. (2012) Formation of Triple-Helical Structures by the 3′-End Sequences of MALAT1 and MENβ Noncoding RNAs. *Proceedings of the National Academy of Sciences of the United States of America* 109(47): 19202-19207. PMCID: PMC3511071
b. **Brown, J.A.**, Bulkley, D., Wang, J., Valenstein, M.L., Yario, T.A., Steitz, T.A. & Steitz, J.A. (2014) Structural Insights into the Stabilization of MALAT1 Noncoding RNA by a Bipartite Triple Helix. *Nature Structural & Molecular Biology* 21(7): 633-640. PMCID: PMC4096706
c. **Brown, J.A.**[#], Kinzig, C.G., DeGregorio, S.J. & Steitz, J.A.[#] (2016) Methyltransferase-Like Protein 16 Binds the 3′-Terminal Triple Helix of MALAT1 Long Noncoding RNA. *Proceedings of the National Academy of Sciences of the United States of America* 113(49): 14013-14018. PMCID: PMC5150381
[#]indicates co-corresponding authors
d. Ruszkowska, A, Ruszkowski, M., Hulewicz, J.P., Dauter, Z. & **Brown, J.A.** (2020) Molecular Structure of a U•A-U-Rich RNA Triple Helix with 11 Consecutive Base Triples. *Nucleic Acids Research* 48(6): 3304-3314. PMCID: PMC7102945

Structure and function of METTL16

Background: Human METTL16 is a recently confirmed m^6A RNA methyltransferase. Understanding the structure and function of METTL16 is an essential step to differentiate it from the major m^6A RNA methlytransferase complex, METTL3/METTL14. Interestingly, METTL16 binds to RNAs with canonical (e.g. double helix in U6 snRNA) and non-canonical (e.g. triple helix in MALAT1) structures.

Findings: We solved X-ray crystal structures of the N-terminal methyltransferase domain (residues 1-291) of human METTL16 in its apo form and in its post-catalytic *S*-adenosylhomocysteine (SAH)-bound state. The methyltransferase domain contains a highly conserved Rossmann fold, a positively charged groove to bind RNA, and unique structural elements that likely contribute to its RNA substrate specificity being distinctly different from the METTL3/METTL14 complex. In addition, our kinetic characterization revealed that METTL16 binds to U6 snRNA (K_{D1} = 18 nM) before SAM (K_{D2} = 126 μM) and exhibits a catalytic efficiency of 4.4×10^{-3} μM^{-1} min^{-1}. However, the MALAT1 triple helix is not methylated by METTL16 under optimized buffer conditions in a test tube. Furthermore, METTL16 binds weakly to the MALAT1 triple helix when it is globally modified with m^6A, pseudouridine or N^1-methylpseudouridine.

Impact: This work provides the foundation for my laboratory to reach our ultimate goal of solving a three-dimensional structure of METTL16 in complex with the MALAT1 RNA triple helix, a complex that may be a gateway into the world of triple-stranded RNA-binding proteins and their triple-helical RNA targets. Additionally, it will also contribute to a better structural understanding of proteins involved in the m^6A epitranscriptome. Interestingly, it is possible that m^6A and pseudouridine modifications in the MALAT1 triple helix may act as a ‘structural switch’ to prevent binding by METTL16.

References:

a. Ruszkowska, A., Ruszkowski, M., Dauter, Z. & **Brown, J.A.** (2018) Structural Insights into the RNA Methyltransferase Domain of METTL16. *Scientific Reports* 8: 5311. PMCID: PMC5871880
b. Breger, K. & **Brown, J.A.** (2023) Elucidating the Kinetic Mechanism of Human METTL16. *Biochemistry* 62(2): 494-506, doi: 10.1021/acs.biochem.2c00601. PMID: 36584291.
c. Schievelbein, M.J., Resende, C., Glennon, M.G., Kerosky, M. & **Brown, J.A.** (2023) Global RNA Modifications to the MALAT1 Triple Helix Differentially Affect Thermostability and Weaken Binding to METTL16. *Journal of Biological Chemistry*, doi: 10.1016/j.jbc.2023.105548. PMID: 38092148.

Altogether, my research efforts have generated 37 publications. A complete list of my publications can be viewed at MyNCBI.