BIOGRAPHICAL SKETCH

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NAME: Woychik, Nancy Ann

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

POSITION TITLE: Professor of Biochemistry and Molecular Biology

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, Madison, WI	B.S.	05/1981	Food Science/Food Chemistry
University of Wisconsin – Madison, Madison, WI	M.S.	05/1984	Microbiology
University of Wisconsin – Madison, Madison, WI	Ph.D.	09/1986	Microbiology
Whitehead Institute, MIT, Cambridge, MA	Postdoctoral	03/1991	Transcription Regulation in Eukaryotes; RNA Polymerase II Structure and Function

A. Personal Statement

The research focus of my laboratory bridges multiple disciplines under the theme of infectious disease: pathogenic mycobacteria including *Mycobacterium tuberculosis* and *Mycobacterium abscessus*, antibiotic resistance/persistence, toxin-antitoxin systems, noncoding RNAs and mechanisms of translation control. We apply a spectrum of state-of-the-art genome-scale transcriptomic/proteomic/metabolomic techniques toward the goals within each discipline and validate these findings using biochemistry, molecular biology, and cell biology. In 2022 we initiated new studies to explore the use of highly selective ribonucleases for targeted cancer therapy.

Ongoing and Completed Research Support from the past three years:

R01 AI154464
“Genome exploration through toxin-mediated ribosome stalling”
Principal Investigator: Nancy Woychik
Agency: NIH/NIAID
Type: RO1
Period: 5/1/20-4/30/24
The goals of this grant are to use several genome-scale tools to understand the molecular mechanisms that underlie *M. tuberculosis* stress survival and also enlist these tools for improved *M. tuberculosis* genome annotation

R01 AI143760
“Transcriptome and proteome remodeling by *Mycobacterium tuberculosis* MazF toxins”
Principal Investigator: Nancy Woychik
Agency: NIH/NIAID
Type: RO1
Period: 12/01/19-11/30/24

The goals of this grant are to investigate the roles of *M. tuberculosis* MazF toxins during stresses relevant to latent tuberculosis

R21 CA267852
"Repurposing *Mycobacterium tuberculosis* tRNase toxins for cancer chemotherapy"
Principal Investigator: Nancy Woychik
Agency: NIH/NCI
Type: R21
Period: 12/01/2021-11/30/2023
The goals of this grants are to perform pilot studies toward development of a novel approach for targeted cancer therapy that enlists highly selective *M. tuberculosis* tRNases to correct overexpression of pro-oncogenic proteins in cancer cells

R21 AI139589
"Molecular triggers of persistent *Mycobacterium abscessus* infections"
Principal Investigator: Nancy Woychik
Agency: NIH/NIAID
Type: R21
Period: 07/01/18-06/30/20 [NCE to 6/30/23]
The goals of this grant are to identify *M. abscessus* genes that influence antimicrobial persistence and resistance

R21 AI15157
"Proteome Reprogramming by tRNA-cleaving toxins"
Principal Investigator: Nancy Woychik
Agency: NIH/NIAID
Type: R21
Period: 2/21/20-1/31/22 [NCE to 1/31/23]
The goals of the grant are to characterize a series of proposed downstream frameshifting events triggered by the activity of tRNA-cleaving toxins

R21 AI135461
"Genome-scale tracking of *Mycobacterium tuberculosis* VapC toxins"
Principal Investigator: Nancy Woychik
Agency: NIH/NIAID
Type: R21
Period: 12/1/17 - 11/30/19 [NCE to 11/30/21]
The goal of this grant is to determine how a subset of *M. tuberculosis* VapC toxins modify the Mtb transcriptome to regulate cell growth

Application #: 556426
"Molecular triggers of persistent *Mycobacterium avium* complex infections in cystic fibrosis"
Principal Investigator: Nancy Woychik
Agency: Cystic Fibrosis Foundation
Type: Research Grant
Period: 4/1/18 - 3/31/20 [NCE to 3/31/21]
The goals of this research are to identify which RNAs are targeted by *Mycobacterium avium* complex (MAC) toxins and how these toxins reshape both the MAC transcriptome and proteome to enhance its persistence and aggravate lung function in cystic fibrosis patients

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B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

July 2013-present RUTGERS UNIVERSITY - ROBERT WOOD JOHNSON MEDICAL SCHOOL
(UMDNJ-RWJMS merges with Rutgers)

	Department of Biochemistry and Molecular Biology Full Professor (with tenure)
July 2012-July 2013	UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL Department of Biochemistry and Molecular Biology Full Professor (with tenure)
July 2001-June 2012	UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL Department of Molecular Genetics, Microbiology & Immunology Associate Professor (with tenure)
1997-present	CANCER INSTITUTE OF NEW JERSEY - Member RUTGERS UNIVERSITY - Graduate School Faculty Member
February 1996-June 2001	UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL Department of Molecular Genetics, Microbiology & Immunology Assistant Professor
April 1991-January 1996	COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK Department of Biological Sciences Adjunct Assistant Professor
April 1991-January 1996	ROCHE INSTITUTE OF MOLECULAR BIOLOGY (closed in Jan. 1996) Department of Biochemistry Assistant Member *Here my research program was fully funded by Hoffman-La Roche yet the institute had a totally academic environment and philosophy*

2023 NIH Bacterial Virulence study section -- *ad hoc* reviewer
2020 NIH Topics in Bacterial Pathogenesis study section -- IDM-B (81) *ad hoc* reviewer
2018 NIH Topics in Bacterial Pathogenesis study section -- IDM-B (80) *ad hoc* reviewer
2016 NIH Topics in Bacterial Pathogenesis study section -- IDM-B (80) *ad hoc* reviewer
2015 The Israel Science Foundation – Reviewer for Research Proposal
2014 The Israel Science Foundation – Reviewer for Research Proposal
2014 The Israel Science Foundation – Reviewer for Research Proposal
2009 The Israel Science Foundation – Reviewer for Research Proposal
2008 The Israel Science Foundation – Reviewer for Research Proposal
2003 The Wellcome Trust – Reviewer for Project Grant Application

Honors

New Jersey Health Foundation Excellence in Research Award 5/20
New Jersey Health Foundation Excellence in Research Award 5/15
New Jersey Cancer Research Award for Scientific Excellence 5/09
Editorial Board-*Molecular and Cellular Biology* Initial term 1997-1999, 2nd term 2000-2002
3rd term 2003- 2005, Final term 2006-2008
American Cancer Society Junior Faculty Award 7/96-6/97
Isermann Family Cancer Research Award (awarded to highest scoring proposal), New Jersey Commission on Cancer Research 6/96-5/98
NIH Postdoctoral Fellowship 1986-1989

C. Contribution to Science

Eukaryotic Transcription (1986-2004)

Starting in the late 1980's as a postdoc, I built this specialty from the ground up having purified yeast *Saccharomyces cerevisiae* RNA polymerase II in large scale, followed by identification and cloning of nearly all of the eukaryotic RNA polymerase subunits when the use of protein sequencing was in its infancy. The sum of this work led to the first complete picture of a eukaryotic RNA polymerase that was immediately exploited by prominent laboratories to do X-ray crystallographic studies on the enzyme. Those structural studies led to two ground-breaking papers (*Cell* 1999, *Science* 2000) from the Kornberg laboratory at Stanford where my key contributions were cited. In 2006, Roger Kornberg was awarded the Nobel Prize for this work.

As an independent investigator I applied the tools developed as a postdoc and undertook the difficult task of functional studies on individual subunits at a time when the rest of the field was focusing on continuing to purify

protein complexes and report the constituents of these complexes (but not their function). Mine was the only laboratory systematically studying RNA polymerase II using the very relevant model system, *S. cerevisiae*. We demonstrated that work in yeast is applicable to higher cells, showing that there was striking structural AND functional conservation to the human and mammalian enzyme. This systematic approach led to several key findings in the field using a thorough set of biochemical, genetic, and molecular modeling experiments. For example, we were able to determine the function of individual subunits (and their domains) and correlate these functions to hallmark features of RNA polymerase.

a. **Woychik, N. A**. and Hampsey, M. (2002) The RNA polymerase II transcription machinery: structure illuminates function. *Cell* 108,453-463 PMID: 11909517 (no PMCID available)
b. **Tan, Q., Linask, K. L.**, Ebright, R. H. and **Woychik, N. A.** (2000) Activation mutants in yeast RNA polymerase II subunit RPB3 provide evidence for a structurally conserved surface required for activation in eukaryotes and bacteria. *Genes Dev.* 14,339-348 PMCID: PMC316356
c. **Miyao, T.** and **Woychik, N. A.** (1998) RNA polymerase subunit RPB5 plays a role in transcriptional activation. *Proc. Natl. Acad. Sci.* 95,15281-15286 PMCID: PMC28034
d. **Hull, M. W., McKune, K.** and **Woychik, N. A.** (1995) RNA polymerase subunit RPB9 is required for accurate start site selection. *Genes Dev.* 9,481-490 PMID: 7883169 (no PMCID available)

*Members of the Woychik laboratory are shown **in bold***

Protein Expression Technology Development (2005-2012)
This work involved the design and optimization of novel protein expression systems in order to enhance success rates for NMR and X-ray crystal structures from selected prokaryotic and eukaryotic recombinant protein targets, including membrane proteins. Through NIH funding from 2006-2010, we had developed a first generation expression system for eukaryotic proteins that has the potential to improve the expression and recovery of correctly folded proteins. This system, called the yeast SPP system, involves expression of the bacterial MazF toxin in *S. cerevisiae* to impart a state of growth arrest. This arrested state allows for continued expression of a recombinant protein without the toxicity that is frequently caused by heterologous protein overexpression (especially for membrane proteins). Another attribute of the yeast SPP expression system is that it enables production of a target protein with virtually no background synthesis of yeast proteins.

We then applied this system to express membrane proteins. Despite serving essential roles in countless important biological processes ranging from receptor-mediated cell signaling to control of cell adhesion, our ability to fully understand the function of membrane proteins is severely hampered by their intrinsic biochemical properties and the low success rates for production using existing protein expression systems. Although structural analysis of proteins has traditionally served as an important complementary approach to classical genetic and biochemical methods for understanding protein function, membrane proteins have been profoundly intractable. The applications for our yeast expression system are multifold and of particular utility for NMR structural genomics and NMR analysis of protein structures under physiological conditions in living cells. **Unfortunately, NIGMS discontinued their commitment for future support for this type of applied project through the Protein Structure Initiative midway through the RO1 funding for this project. In fact, there is no longer a Protein Structure Initiative at NIH.** Therefore, I shifted the focus of the laboratory to current projects and did not have the luxury to publish our substantial data on these novel yeast expression approaches.

a. Suzuki, M., Zhang, J., **Liu, M.**, **Woychik, N. A.** and Inouye, M. (2005) The use of an mRNA interferase for single protein production in living cells. *Mol. Cell* 18,253-261 PMID: 15837428 (no PMCID available) (highlighted in *The Scientist*, *Chemical Engineering News* and *Genetic Engineering News*)
b. Suzuki, M., **Roy, R.**, Zheng, H., **Woychik, N. A.** and Inouye, M. (2006) Bacterial bioreactors for high yield production of recombinant protein. *J. Biol. Chem.* 281,37559-37665. PMID: 17020876 (no PMCID available)

*Members of the Woychik laboratory are shown **in bold***

Structure and Function of Bacterial Toxin-Antitoxin Systems (2004-2014)
The primary focus of this work was to build a foundation for the field since in 2003 the function of only a few TA system toxins were known. We used two model systems, *E. coli* and *Clostridium difficile* to better understand the basic functions of these specialized toxins. We were the first to identify the general function of the Doc, HigB and YafQ toxins, the first to pinpoint the activity of the only MazF toxin in *C. difficile*, corrected the sequence specificity and function of the RelE toxin, and along with a competitor whose paper came out while ours was under review in a high visibility journal, we discovered that the Doc toxin is an EF-Tu kinase.

a. **Cruz J. W., Rothenbacher, F. P.**, Maehigashi, T., Lane, W. S., Dunham, C. M. and **Woychik, N. A**. (2014) Doc toxin is a kinase that inactivates elongation factor Tu. *J. Biol. Chem.* 289,7788-7798 PMCID: PMC3953291
b. **Hurley, J. M., Cruz, J. W**., Ouyang, M. and **Woychik, N. A.** (2011) Bacterial toxin RelE mediates frequent codon-independent mRNA cleavage from the 5′ end of coding regions *in vivo. J. Biol. Chem.* 286,14770–14778 PMCID: PMC3083149
c. **Prysak, M. H., Mozdzierz, C. J., Cook, A. M.**, Zhang, Y., Inouye, M. and **Woychik, N. A.** (2009) Bacterial toxin YafQ is an endoribonuclease that associates with the ribosome and blocks translation elongation through sequence-specific and frame-dependent mRNA cleavage. *Mol. Microbiol.* 71,1071-87 PMID: 19210620 (no PMCID available)
d. **Liu, M.**, Zhang, Y., Inouye, M. and **Woychik, N. A.** (2008) Bacterial Addiction Module Toxin Doc Inhibits Translation Elongation through its Association with the 30S Ribosome. *Proc. Natl. Acad. Sci. USA* 105,5885-5890 PMCID: PMC2311363

*Members of the Woychik laboratory are shown **in bold***

***Mycobacterium tuberculosis* Toxin-Antitoxin Systems (2010-present)**

I am applying my expertise in biochemistry, genetics and structure-function to better understand how TA toxins affect growth, stress survival and virulence in Mtb where the persistence of the organism is intrinsic to its pathogenicity. We have made several seminal contributions to the field. We discovered that some Mtb MazF toxins cleave rRNA, debunking the widely held belief that MazF toxins are primarily “mRNA interferases”. We developed a powerful 5’ RNA-seq approach that produces an accurate, comprehensive snapshot of *tuberculosis* RNA targets of endoribonuclease toxins. We were the first to determine that an Mtb VapC toxin is an isoacceptor-specific tRNase, a function not previously assigned to any Mtb TA toxin. We uncovered a new mode of regulation by tRNA-cleaving toxins that enlists highly selective ribosome stalling to reprogram Mtb physiology. Most recently, we reported that VapC4 reprograms Mtb metabolism to specifically defend against oxidative and copper stresses from macrophages upon infection. This is the first example of a TA toxin playing a direct and vital role in activating two critical stress response pathways essential for Mtb viability during infection.

a. **Barth, V. C., Chauhan, U.,** Zeng, J.-M., Su, X., Zheng, H., Husson, R.N. and **Woychik, N. A.** (2021) *Mycobacterium tuberculosis* VapC4 toxin engages small ORFs to initiate an integrated oxidative and copper stress response *Proc. Natl. Acad. Sci. USA* 118(32):e2022136118. PMCID: PMC8364209
b. **Barth, V. C.**, Zeng, J.-M., Vvedenskaya, I.O., Ouyang, M., Husson, R.N. and **Woychik, N. A.** (2019) Toxin-mediated ribosome stalling reprograms the *Mycobacterium tuberculosis* proteome. *Nat. Commun*. 10, 3035. PMCID: PMC6620280
c. **Cruz, J.W., Sharp, J.D.**, Hoffer, E.D., Maehigashi, T., Vvedenskaya, I.O., Konkimalla, A., Husson, R.N., Nickels, B.E., Dunham, C.M., and **Woychik, N. A.** (2015) Growth-regulating *Mycobacterium tuberculosis* VapC-mt4 toxin is an isoacceptor-specific tRNase. *Nat. Commun*. 6,7480. PMCID: PMC4620994
d. **Schifano, J. M.**, Vvedenskaya, I. O., Knoblauch, J. G., Ouyang, M., Nickels, B. E., and **Woychik, N. A.** (2014). An RNA-seq method for defining endoribonuclease cleavage specificity identifies dual rRNA substrates for toxin MazF-mt3. *Nat. Commun.* 5,3538 PMCID: PMC4090939

*Members of the Woychik laboratory are shown **in bold***

Complete List of Published Work in MyBibliography:
https://pubmed.ncbi.nlm.nih.gov/?term=woychik+n