BIOGRAPHICAL SKETCH

NAME: **Christine M. Dunham, Ph.D.**

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

POSITION TITLE: Professor of Chemistry

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Barnard College, Columbia University, New York, NY	B.A.	09/1993	05/1997	Biochemistry
University of California, Santa Cruz, CA	Ph.D.	09/1997	06/2003	Structural Biology
MRC Laboratory of Molecular Biology, Cambridge, UK	Postdoc	01/2004	04/2008	Structural Biology

A. Personal Statement

My role in this NCCAT BAG proposal is to solve structures of ribosomes through the following three subprojects: 1) Basis for antibiotic resistance mechanisms and uncovering pathways to new antimicrobials; 2) how RNA modifications and quality control pathways contribute to overall ribosome fidelity; and 3) how ribonucleases inhibit translation by specific degradation of ribosomes or tRNAs.

The Dunham lab research centers on understanding how stress alters translation, the mechanism of ribosome dysregulation, and the roles of toxin-antitoxin pairs in inhibiting protein synthesis to alter bacterial physiology. Starting as a graduate student in the laboratory of Dr. William G. Scott (at the University of California, Santa Cruz) and then as an American Cancer Society Postdoctoral Fellow in the laboratory of Dr. Venki Ramakrishnan at the MRC Laboratory of Molecular Biology in Cambridge, England. In my independent research group at Emory University, my lab studies the structure, function and regulation of the bacterial ribosome and how bacterial protein toxins regulate translation. Over the course of fourteen years, I have expanded my research interests to projects centered on the molecular basis for how stress regulates translation and the roles of toxin-antitoxin pairs in inhibiting protein synthesis to alter bacterial physiology using molecular biology, biochemistry, X-ray crystallography and single particle cryo-electron microscopy (cryo-EM).

Select current research support

NIH/NIGMS R01 GM093278; Dunham (PI), No Cost Extension 09/01/2019–08/31/2023
Molecular basis of ribosomal frameshifting.

NIH/NIAID R01 AI088025; Conn, Dunham (MPI) 06/01/2020–05/30/2025
RNA modification and antibiotic resistance.

NIH/NIGMS R01 GM121650; Dunham, Keiler (MPI) 08/24/2022–08/23/2026
Ribosome rescue.

NIH/NIAID R01 AI158706-01A1; Baugh, Keiler (MPI) 11/01/2021–10/31/2026
Targeting trans*-translation to kill* M. tuberculosis *non-replicating persister cells*

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023– Professor, Dept of Chemistry, Emory University, Atlanta, Georgia.
2021–2023 Professor, Dept of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.
2017–2021 Associate Prof, Dept of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.
2008–2016 Assistant Prof, Dept of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.

2004–2008 American Cancer Society Postdoctoral Fellow, MRC Laboratory of Molecular Biology, Cambridge, UK. Advisor: Group Leader Venki Ramakrishnan.
2004 Medical Research Council Career Development Fellow, MRC Laboratory of Molecular Biology, Cambridge, UK. Advisor: Group Leader Venki Ramakrishnan.
1996 NSF Summer Undergraduate Research Fellow, University of Texas Medical Branch at Galveston. Advisor: Professor Bennett Van Houten.
1994–1995 NSF Summer Undergraduate Research Fellow, Albany Medical College, Albany, New York. Advisor: Professor Peter Weber.

Awards/Honors

2022 American Society of Biochemistry and Molecular Biology (ASBMB) fellow
2022 Kavli Fellow, National Academy of Sciences
2021 Emory School of Medicine Innovation for Impact Award
2021–2022 Chair, Molecular Genetics A (MGA) Study Section
2018–2022 NIH Permanent Study Section Member, Molecular Genetics A (MGA)
2018 Cozzarelli Prize, National Academy of Sciences, Best Biological Sciences paper
2018 American Society of Biochemistry and Molecular Biology (ASBMB) Young Investigator
2017 American Crystallographic Association Etter Early Career Awardee
2016–2021 Burroughs Wellcome Investigator in the Pathogenesis of Infectious Diseases
2011–2015 Pew Scholar in the Biomedical Sciences
2010–2015 NSF Early Career Development (CAREER) Award
2003 Best Poster Prize, Gordon Research Conference on Nucleic Acids (Ph.D.)
1999–2003 NSF-GAANN Graduate Research Fellowship

Other Experience, Service and Professional Memberships

2023–present Editorial Board Member, *Nucleic Acids Research*
2023–present Chair, Awards Committee, American Society of Biochemistry and Molecular Biology (ASBMB)
2022–present Chair, Awards Committee, RNA Society
2020–present Publications Committee, American Society of Biochemistry and Molecular Biology (ASBMB)
2018–present Editorial Board Member, *Journal of Biological Chemistry*
2018–present Editorial Board Member, *Molecular Microbiology*
2018–2023 NIH Permanent Study Section Member, Molecular Genetics A (MGA)
2016 Faculty mentor, GRC Microbial Stress Responses, Mt Holyoke, MA.
2015 2016 Conference Organizing committee, ASBMB, San Diego, CA.
2013 Pew Charitable Trusts 2014 Conference organization committee, Chile.
2012 Session chair, “Supramolecular Assemblies”, American Crystallographic Association conference, Honolulu, HI.
2011 Conference organizing committee, Suddath symposium on the Ribosome, Institute for Bioengineering & Bioscience, Georgia Tech, Atlanta, GA.
2008–present Manuscript reviewer: *Nature, Science, PNAS, Cell, Molecular Cell, Nucleic Acids Research*, *Structure*, *J. Biol. Chem., Biochemistry*, *Biophysical Journal, Molecular Microbiology, Nature Structure & Molecular Biology*, *Journal of Bacteriology*, *Journal of American Chemistry Society*, *RNA*, *PLoS Genetics, Scientific Reports, Nature Chemical Biology, PLoS ONE.*
2008–present Temporary grant reviewer/study section: NIH K99 Pathways to Independence Awards study section, Macromolecular Structure and Function C (MSFC) grant study section, ZRG1 Biological Chemistry and Macromolecular Physics; American Heart Association, Basic Cell Protein and Crystallography grant study section; NSF, Division of Molecular and Cellular Biosciences, CAREER, Graduate Student Research Fellowship study sections; American Cancer Society, RNA Mechanisms of Cancer grant study section.
2001–present RNA Society (since 2005), American Crystallographic Association (since 2001), Biochemical Society UK (2004-2007), American Society for Microbiology (ASM; since 2008), and The American Society for Biochemistry and Molecular Biology (ASBMB; since 2011).

C. Contributions to Science

Complete list of publications in My NCBI:
https://www.ncbi.nlm.nih.gov/myncbi/1hI1rm7vAMckt/bibliography/public/

1. *Ribosomal mRNA frameshifting.* mRNA frameshifting controls gene expression in that the noncanonical reading of the genetic code facilitates expression of different protein products. Frameshift-prone tRNAs and mRNAs that contain complex RNA tertiary structures are two major causes for the change in the mRNA reading frame. To study this, we focused on factors identified in genetic suppressor studies or naturally occurring defects and/or mutations. As a postdoctoral fellow, I solved the first structure of a frameshift suppressor tRNA bound to the 30S decoding center (PMCID: PMC1869038). These studies provided an alternative model for how tRNAs facilitate a change in the reading frame. In my own lab, we have extended these initial observations by solving a number of different frameshift-prone tRNAs bound to the 70S ribosome that have defined how additional tRNA nucleotides and modifications in the anticodon loop regulate the mRNA reading frame (**a,b**). Further, we discovered how tRNA modifications maintain the mRNA frame and how dysregulation results in the ribosome losing its grip on the mRNA (**c,d**).

a. Maehigashi T*, Dunkle JA*, Miles SJ, Dunham CM (2014) Structural insights into +1 frameshifting promoted by expanded or modification-deficient anticodon stem-loops. *Proc Natl Acad Sci* 111(35):12740-5. PMCID: PMC4156745. *These authors contributed equally.
b. Hong S*, Sunita S*, Dunkle JA, Maehigashi T, Dunham CM (2018) Mechanism of +1 tRNA-mediated frameshifting. *Proc Natl Acad Sci* 115(44):11226-31. PMCID: PMC6217423. *These authors contributed equally.
    Commentary by JF Atkins. Culmination of a half-century quest reveals insight into mutant tRNA-mediated frameshifting after tRNA departure from the decoding site. *Proc Natl Acad Sci* 115(44):11221-23. PMCID: PMC6217412
c. Nguyen HA, Hoffer ED, Dunham CM (2019) Importance of tRNA anticodon loop modification and a conserved, noncanonical anticodon stem pairing in $tRNA^{Pro}$-CGG for decoding. *Journal of Biological Chemistry* 294(14):5281-91. PMCID: PMC6462517. *Selected as the Editor's Pick, an honor bestowed on the top 2% of JBC papers.*
d. Hoffer ED, Hong S, Sunita S, Whitford P, Gonzalez RL Jr, Dunham CM (2020) Structural insights into mRNA reading frame regulation by tRNA modification and slippery codon-anticodon pairing. *eLife*. 9:e51898. PMCID: PMC7577736.

2. *Molecular basis of translational dysregulation.* Biological fitness is critically dependent upon the accurate flow of genetic information. Although proofreading mechanisms exist, errors still occur and this breakdown in translational fidelity is detrimental to cells. Miscoding or misreading of the genetic code can occur with high frequency with specific mRNA-tRNA pairs and the basis of this dysregulation is not currently understood at the molecular level. We first studied this question using 16S rRNA *ribosome ambiguity mutants* (*ram*), a known hyperaccurate phenotype, as a model system. We identified global changes of these *ram* ribosomes that were allosterically communicated to the decoding center providing a molecular basis of this hyperaccurate phenotype (**a**). The molecular basis for why specific mRNA-tRNA pairs are more prone to miscoding has also been an elusive question despite many structures solved that show very little differences between the decoding of cognate as compared to near-cognate mRNA-tRNA pairs. We took a different approach and studied the biochemically well-studied $tRNA^{Ala}$. We found that the ribosome identifies correct from in correct mRNA-tRNA pairing by directly interacting with the anticodon stem of correct pairs (**b**). These studies provide insight, for the first time, into how tRNA stability and recognition by the ribosome can lead to accurate decoding. **(c)** Near-cognate mRNA-tRNA pairs that lead to activation of the post-peptidyl quality control pathway result in a loss of ribosome fidelity at the decoding center. We determined that near-cognate mispairings that have bypassed ribosome fidelity mechanisms, disrupt the mRNA path in the decoding center leading to a loss of fidelity. This ensures that incorrect tRNAs and release factors can bind to the ribosome and halt translation. (**d**) Defects in the rescuing of bacterial ribosomes that have stalled due to issues with mRNAs (5-10% of all cellular mRNAs) lead to cell death. Therefore, the ability to inhibit rescue was identified as a possible new antimicrobial target and compounds that specifically inhibit ribosome have been identified. In collaboration with the Keiler lab, we determined the molecular basis of action of one identified compound that inhibits rescue and specifically, we solved cryo-EM structures of the drug bound to a stalled ribosome.

a. Fagan CE, Dunkle JA, Maehigashi T, Dang MN, Deveraj A, Miles SJ, Qin D, Fredrick K, Dunham CM (2013) Reorganization of an intersubunit bridge induced by disparate 16S ribosomal ambiguity mutations mimics an EF-Tu-bound state. *Proc Natl Acad Sci* 110(24):9716-21. PMCID: PMC3683721.
    Commentary by PB Moore. Ribosomal ambiguity made less ambiguous. *Proc Natl Acad Sci* 110(24):9627-8. PMCID PMC3683732.

b. Nguyen HA, Sunita S, Dunham CM (2020) Disruption of evolutionarily conserved tRNA elements impairs accurate decoding. *Proc Natl Acad Sci* 117(28):16333–38. PMCID: PMC7368331.
c. Nguyen HA, Hoffer ED, Maehigashi T, Fagan CE, Dunham CM (2023) Structural basis for reduced ribosomal A-site fidelity in response to P-site codon-anticodon mismatches. *Journal of Biological Chemistry,* 299(4):104608. PMCID: PMC10140155.
d. Aron ZD*, Mehrani A*, Hoffer ED*, Connolly KL, Torhan MC, Alumasa JN, Srinivas P, Cabrera M, Hosangadi D, Barbor JS, Cardinale S, Kwasny S, Butler M, Opperman T, Bowlin T, Jerse A, Stagg SM, Dunham CM[#], Keiler KC[#] (2021) Ribosome rescue inhibitors clear *Neisseria gonorrhoeae in vivo* using a new mechanism. *Nature Communications.* 12(1):1799. PMCID: PMC7979765. [#]Co-corresponding authors.

3. *Role of Modifications in Protein Synthesis*. Modifications to ribosomal RNA and proteins can tune protein synthesis or in other cases, modifications by pathogens are an antimicrobial strategy to gain resistance. In the latter case in collaboration with the Conn lab, we determined the molecular basis for recognition of a complex RNA tertiary structure within the context of the intact 30S subunit by a pathogen-derived aminoglycoside-resistance rRNA methyltransferase (**a**). These studies were the first of a modification enzyme bound to a ribosome and helped rationalize why an intact 30S subunit was required for recognition by this family of enzymes. We further characterize interactions of a different enzyme family and showed the diverse macromolecular recognition by divergent family members (**b,c**). In collaboration with the Ibba lab, we identified the molecular basis for how oxidative stress in Salmonella enterica serovar Typhimurium causes a tRNA synthetase to become more accurate to combat changing levels of amino acids (**d**).

a. Dunkle JA, Vinnal K, Desai PM, Zelinskaya N, Savic M, West DM, Conn GL[#,] Dunham CM[#] (2014) Molecular recognition and modification of the 30S ribosome by the aminoglycoside-resistance methyltransferase NpmA. *Proc Natl Acad Sci* 111(17):6275-80. PMCID: PMC4035980. [#]Co-corresponding authors.
b. Srinivas P, Nosrati M, Zelinskaya N, Dey D, Comstock LR, Dunham CM[#], Conn GL[#] (2023) 30S subunit recognition and G1405 modification by the aminoglycoside-resistance 16S ribosomal RNA methyltransferase RmtC. *Proceedings of the National Academy of Sciences.* 120(25):e2304128120 PMID: 37307464. [#]Co-corresponding authors.
c. Nosrati M, Dey D, Mehrani A, Strassler SE, Zelinskaya N, Hoffer ED, Stagg SM, Dunham CM, Conn GL (2019) Functionally critical residues in the aminoglycoside resistance-associated methyltransferase RmtC play distinct roles in 30S substrate recognition. *Journal of Biological Chemistry* 294(46):17642-53. PMCID: PMC6873201
d. Srinivas P, Steiner RE, Pavelich IJ, Guerrero-Ferreira R, Juneja P, Ibba M, Dunham CM (2021) Oxidation alters the architecture of the phenylalanyl-tRNA synthetase editing domain to confer hyperaccuracy. *Nucleic Acids Research* 49(20):11800-11809.

4. *Role of bacterial toxin-antitoxin modules.* Bacteria quickly adapt to changing environmental conditions by altering their gene expression to facilitate survival. My laboratory has investigated the roles that toxin-antitoxin pairs play in this transition. Most toxins inhibit protein synthesis, and my laboratory has been focused on the largest class of translational inhibitors, ribosome-dependent toxins. These toxins recognize and cleave mRNA bound to the ribosome. We identified the *E. coli* YafQ toxin features required for ribosome binding and mRNA catalysis that distinguishes these specialized RNases from general microbial RNases (**a**). In contrast to the prevailing view that bacterial toxins are global translational inhibitors, we demonstrated that the ribosome dependent HigB toxin only cleaves specific mRNA transcripts which suggests a more specialized role in the regulation of protein synthesis (**b**). To study a bacterial toxin specifically activated during thermal stress, we focused on the *E. coli* YoeB toxin that uniquely adopts a dimeric oligomeric state. Using biochemistry and structural biology approaches, we determined that its dimeric role is not required for activity but rather, simply is needed to withstand elevated temperatures (**c**). Another interest we have is in understanding the proteolysis of antitoxins or antidote proteins that occurs during stress to release its cognate toxin. We identified the importance of the C-terminus of the *E. coli* DinJ antitoxin required for its recognition by the Lon protease (**d**).

a. Maehigashi T*, Ruangprasert A*, Miles SJ, Dunham CM (2015) Molecular basis of ribosome regulation and mRNA hydrolysis by the *E. coli* YafQ toxin. *Nucleic Acids Res* 43(16):8002-12. PMCID: PMC4652777. *These authors contributed equally.
b. Schureck MA, Dunkle JA, Maehigashi T, Miles SJ, Dunham CM (2015) Defining the mRNA recognition signature of a bacterial protein toxin. *Proc Natl Acad Sci* 112(45):13862-7. PMCID: PMC4653167.

c. Pavelich IJ*, Maehigashi T*, Ruangprasert A, Hoffer ED, Miles SJ, Dunham CM. (2019) Monomeric YoeB toxin retains RNase activity but adopts an obligate dimeric form for thermal stability. *Nucleic Acids Research* 47(19):10400-13. PMCID: PMC6821326. *These authors contributed equally.
d. Ruangprasert A, Maehigashi T, Miles SJ, Dunham CM (2017) Importance of the *E. coli* DinJ antitoxin carboxy terminus for toxin suppression and regulated proteolysis. *Mol Micro* 104(1):65-77.

5. Pioneering structural studies of ribosome function. Protein synthesis is carried out by the ribosome and is one of the most conserved biological processes. As a postdoctoral fellow in Venki Ramakrishnan's lab, I solved the first high-resolution structure of the entire bacterial ribosome (**a**). This work continues to impact the field and has been cited >950 times. Although this methodology helped push the field forward, the most significant biological achievement has been the mechanistic insights such structures have revealed, including how translation factors facilitate termination and recycling (**b**), how GTPase elongation factors modulate activity (**c**), and how bacterial toxins target the ribosome during the stringent response (**d**).

a. Selmer M*, Dunham CM*, Murphy IV FV, Weixlbaumer A, Petry S, Kelley AC, Weir J, Ramakrishnan V (2006) Structure of the 70S Ribosome Complexed with mRNA and tRNA. *Science* 313(5795):1935-42. *These authors contributed equally.
b. Weixlbaumer A, Petry S*, Dunham CM*, Selmer M*, Kelley AC, Ramakrishnan V (2007) Crystal structure of the ribosome recycling factor bound to the ribosome. *Nat Struct Mol Biol* 14(8):733-7. *These authors contributed equally.
c. Gao Y-G, Selmer M, Dunham CM, Weixlbaumer A, Kelley AC, Ramakrishnan V (2009) The Structure of the Ribosome with Elongation Factor G Trapped in the Posttranslocational State. *Science* 326(5953):694-99. PMCID: PMC3763468.
d. Neubauer C*, Gao, Y-G*, Andersen KR*, Dunham CM, Kelley AC, Hentschel J, Gerdes K, Ramakrishnan V, Brodersen DE (2009) The structural basis for mRNA recognition and cleavage by the ribosome-dependent endonuclease RelE. *Cell* 139(6):1084-1095. PMCID: PMC2807027. *These authors contributed equally.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Conn, Graeme Leslie

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

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Edinburgh, UK	B.Sc.	05/1993	Chemistry
University of Edinburgh, UK	Ph.D.	10/1996	Chemistry
Johns Hopkins University	Postdoc	12/1999	Structural Biology

A. Personal Statement

My role on this request for BAG access to the NCCAT Krios microscope facility will be to serve as a co-applicant with Dr. Dunham and as a sub-project leader. The specific areas my lab will focus on are the determination of ribosome subunit-rRNA methyltransferase complexes and methylated ribosome-drug complexes and determination of Trm10 family members bound to substrate tRNAs. A major research focus of my group over the last ~20 years has been to define the molecular basis for resistance to ribosome-targeting antibiotics arising from rRNA methylation. Other projects on tRNA and protein methylation, and innate immune protein regulation by RNA have developed from these earlier interests in RNA structure, modification, and function. On-going projects in my lab are currently funded by the NIH as follows: ***i)*** ribosomal RNA methyltransferase enzymes related to bacterial resistance to antibiotics or virulence and/ or human health (NIH/NIAID R01 AI088025–also see details below); ***ii)*** tRNA modification by the Trm10 family of SPOUT methyltransferases (NIH/NIGMS R01 GM130135); ***iii)*** non-coding RNA structure and activity against proteins of the human innate immune response (NIH/NIAID AI144067). Finally, we are currently seeking funding for new work on bacterial efflux systems in studies that developed from our on-going work in the area of bacterial antibiotic resistance mechanisms (previously supported by Emory university seed grant programs). As such, we have an established track-record of using a broad array of approaches including biochemistry, structural (now, typically cryo-EM) and other biophysical methods, molecular biology and microbiology to dissect fundamental biological mechanisms related to this topic. These efforts will also be supported by Emory's EM core (of which I am one of three Scientific Co-directors) as well as colleagues among Emory's major users of this facility. Determining the structures proposed in this BAG application will represent a major advance in our understanding of how these RNA modification enzymes carry out their function in relation to antibiotic resistance and tRNA biology, and, more generally, about how rRNA modification enzymes recognize and site-specifically modify their substrates.

Selected current research support (as noted above):

1. NIH/NIAID, R01 AI088025, *RNA modification and antibiotic resistance,* MPI–Conn*,Dunham (5/1/2010–4/30/2025).
2. NIH/NIGMS, R01 GM130135, *Mechanisms and biological functions of SPOUT methyltransferases*, MPI-Jackman*, Conn (09/14/2018–07/31/2023). [Pending admin review: renewal application has been recommended for funding by PO]
3. NIH/NIAID, R01 AI144067, *dsRNA regulation of the cytosolic innate immune system*, PI–Conn, CoI–Lowen, (3/12/2019–2/28/2028).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020–present Co-scientific Director, Robert P. Apkarian Integrated Electron Microscopy Core, Emory University

2019–present Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.
2008–2019 Associate Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.
2007–2008 Senior Lecturer (equivalent to Associate Professor, *with tenure*), Faculty of Life Sciences, University of Manchester, UK.
2000–2007 Lecturer (equivalent to Assistant Professor, *tenure track*), Dept. Biomolecular Sciences, UMIST (2000-2004)/ Faculty of Life Sciences, University of Manchester, UK (2004-2007).
2000–2004 Wellcome Trust Independent Research Career Development Fellow, Dept. Biomolecular Sciences, UMIST, UK.
1999–2000 Wellcome Trust Postdoctoral Fellow, Dept. of Biomolecular Sciences, UMIST, UK.
1996–1999 Wellcome Trust Postdoctoral Fellow, Johns Hopkins University, Baltimore, MD.

Awards/Honors

2019 Researcher Appreciation Day recognition, Emory University School of Medicine.
2018 Hidden Gem award, Emory University School of Medicine.
2000–2004 Wellcome Trust Independent Research Career Development Fellowship.
1996–2000 Wellcome Trust International Traveling Prize Fellowship (Postdoctoral).
1993–1996 Royal Society of Edinburgh Caledonian Trust Scholarship (PhD).
1990–1994 1st, 3rd and 4th Year Undergraduate Class Prize (top ranked student), Department of Chemistry, University of Edinburgh, UK.
1990–1993 Faculty of Science and Engineering Bursary, University of Edinburgh, UK.

Other Experience, Service and Professional Memberships

2022-present Associate Editor, npj Antimicrobials and Resistance.
2019 NIH/NIAID ZAI1 LR-M (M1), Special Emphasis Panel (RFA-AI-18-025, Elucidating the Functional Roles of Non-Coding RNAs in Viral Infectious Diseases (R21).
2017-2019 Guest Editor, special topic "*Bacterial Mechanisms of Antibiotic Resistance: A Structural Perspective*", Frontiers in Molecular Biosciences.
2015-present NIH/ CSR F13, Fellowships: Infectious Diseases and Microbiology (five times total, most recently Nov 2020).
2014-present Frontiers in Molecular Biosciences, Reviewing Editor (Structural Biology).
2013-2014 Chair, Proteins and Crystallography Committee 2, American Heart Association.
2012 Co-chair, Proteins and Crystallography Committee 2, American Heart Association.
2012 'Recombinant and *in vitro* RNA synthesis: Methods and Protocols' (Editor), Methods in Molecular Biology series (Walker, J.M., series Editor), Humana Press.
2001-present *Ad hoc* grant reviewer/ study section: The Wellcome Trust, BBSRC (UK), American Heart Association, American Cancer Society, NIH/CSR (IMST-G 30 (S10 Shared Equipment review), ZRG1 BCMB-R 02 M, ZDC1 SRB-K17 (R03 review) and DP5 Director's Early Independence Award), American Heart Association and the Canadian Council for the Arts (Killam Research Fellowship).
2001-present Manuscript reviewer: *Nature Methods, Nature Protocols, PNAS, J.Mol. Biol.*, *Nucleic Acids Res.*, *J. Biol. Chem., BioTechniques*, *Biochemistry*, *Cell. Mol. Life Sci., Chem. Senses*, *Biol. Cell., J. Biotechnology, Current Biology, Molecular Microbiology, FEMS Letters and PLoS-ONE, ChemBioChem., RNA-Journal of the RNA Society, Frontiers Microbiology, mBio.*
2000-present Biochemical Society UK (2003-2007), Association for Chemoreception Sciences (2003-2011), American Society for Microbiology (ASM; since 2008), American Association for the Advancement of Science (AAAS; since 2009), American Crystallographic Society (ACA; since 2010), The American Society for Biochemistry and Molecular Biology (ASBMB; since 2011), and the RNA Society (since 2017).

C. Contributions to Science

Complete List of Published Work in My NCBI (75 total publications): My Bibliography-Conn, G.L.
http://www.ncbi.nlm.nih.gov/sites/myncbi/graeme.conn.1/bibliography/40548662/public/?sort=date&direction=descending

Trainees (my lab) are highlighted in the references: **postdoctoral researchers** (bolded and underlined;) and *graduate students* (italicized and underlined; also: †rotation student only, #undergraduate); additionally, trainees from minorities underrepresented in the sciences are noted¶.

1. Bacterial ribosomal RNA modification and antibiotic resistance. Aminoglycoside antibiotics typically act

by binding and inducing specific conformational changes in the ribosome "decoding center" that result in aberrant protein synthesis. Aminoglycosides have retained potent activity, leading to a reevaluation of their potential utility in the clinic in the face of increasing resistance to many first line drugs. Clinical aminoglycoside resistance typically arises through the action of aminoglycoside modifying enzymes, whereas drug-producing bacteria also use 16S rRNA methyltransferase enzymes to modify the ribosomal drug binding site, either at the N7 position of G1405 (m^7G1405) or the N1 position of A1408 (m^1A1408). Now, a serious threat to the future clinical usefulness of aminoglycosides has arisen from the acquisition and spread among human bacterial pathogens of these rRNA methyltransferases. rRNA modification confers exceptionally high-level resistance and, combined, these modifications are capable of blocking the effects of *all* clinically useful aminoglycosides including the latest generation of drugs. Determining the structures of these enzymes and defining the features which govern their interactions with cosubstrate S-adenosyl-L-methionine (SAM) and 30S substrate, have been a major contribution from my lab. Our early work defined critical features for SAM binding and 30S recognition by Sgm (a drug producer m^7G1405 enzyme) and, subsequently, revealed the first structures of m^1A1408 enzymes with cosubstrate from both aminoglycoside-producing and human pathogenic bacteria. Next, in collaboration with Christine Dunham's group (Emory, Chemistry) we presented a breakthrough in the field with the determination of a first structure of a resistance methyltransferase (NpmA) bound to its 30S substrate (**ref. a**). This work was followed by a detailed mechanistic study to dissect the molecular basis of 30S recognition by NpmA. Together, these publications revealed the basis for the requirement of mature 30S as substrate, and the molecular details underpinning specific target recognition, including flipping of the target A1408 base into the NpmA active site. Our on-going studies include complementary studies of members of the clinically more prevalent m^7G1405 family (e.g. **ref. b,d**) as well as the dual (30S and 50S subunit) methyltransferase TlyA from *Mycobacterium tuberculosis* (e.g. **ref. c**), which is associated with mycobacterial resistance to the tuberactinomycin drugs capreomycin and viomycin. Our long-term goal is to exploit the understanding we develop of these methyltransferase enzymes and their target recognition mechanisms to facilitate development of specific inhibitors of these resistance determinants.

1a. **Dunkle, J.A**., *Vinal, K*., **Desai, P.M**., Zelinskaya, N., *Savic, M.*, **West, D.M.¶**, *Conn, G.L. and *Dunham, C.M. (2014). Molecular recognition and modification of the 30S ribosome by the aminoglycoside-resistance methyltransferase NpmA. *Proc. Natl. Acad. Sci. U.S.A.* **111**(17), 6275-6280. (*Co-corresponding author) [PMCID: PMC4035980]

1b. **Nosrati, M**., **Dey, D**., Mehrani, A., D. *Strassler, S.E*., Zelinskaya, N., Hoffer, E.D., Stagg, S.M., Dunham, C.M. and Conn, G.L. (2019). Functionally critical residues in the aminoglycoside resistance-associated methyltransferase RmtC play distinct roles in 30S substrate recognition. *J. Biol. Chem. 294, 17642-17653.* [PMCID-PMC6873201]

1c. *Laughlin, Z.T*., **Nandi, S**., **Dey, D**., Zelinskaya, N., **Witek, M.A**., Srinivas, P., Nguyen, H.A., *Kuiper, E.G*., Comstock, L.R., Dunham, C.M. and Conn, G.L. (2022) 50S subunit recognition and modification by the Mycobacterium tuberculosis ribosomal RNA methyltransferase TlyA. *Proc. Natl. Acad. Sci. U. S. A.*, **119**, e2120352119. [PMCID: PMC9168844]

1d. Srinivas, P., **Nosrati, M**., Zelinskaya, N., **Dey, D**., Comstock, L.R., Dunham, C.M.* and Conn, G.L.* (2023). 30S subunit recognition and G1405 modification by the aminoglycoside-resistance 16S ribosomal RNA methyltransferase RmtC, *Proc. Natl. Acad. Sci. USA,* **120**(25):e2304128120. doi: 10.1073/pnas.2304128120.). *Co-corresponding authors *Co-corresponding authors

2. Structure, activity, and substrate selection by the *P. aeruginosa* RND pump MexXY-OprM. My lab's work on the resistance-nodulation-cell division (RND) family efflux pumps represents a relatively new direction in our fundamental studies of bacterial antibiotic resistance. These studies originated from on-going *in silico* analyses of aminoglycoside antibiotic dynamics and interactions with *methylated* 30S ribosome subunits to understand how some drugs of this class appear to be able to "evade" the effects of RNA modification by the aminoglycoside-resistance 16S rRNA methyltransferases (see **Contribution 1** above). Our current focus is on *P. aeruginosa* MexXY-OprM which is unique in both its dependence on aminoglycosides for regulation of its expression and its ability to preferentially efflux these antibiotics leading to clinical resistance (e.g. in strains isolated from individuals with cystic fibrosis). Using phylogenetic analyses, computational molecular modeling of the MexXY-OprM complex, and *in silico* ligand docking in the MexY distal binding pocket (DBP) we proposed a novel hypothesis: that the physicochemical properties of the MexY DBP have evolved to prefer aminoglycosides over β-lactams via a "Goldilocks binding affinity" (**ref. a**). That is, substrates (aminoglycosides) must bind sufficiently tightly to be taken up by MexY but not too tightly, so as to impede movement through the transporter to the adaptor protein MexX. Supporting this novel concept, β-lactams are universally predicted to bind more tightly to MexY, and the

converse is also true: aminoglycosides were predicted, without exception, to bind more tightly to MexB, of the homologous RND pump MexAB-OprM which exports β-lactams but not aminoglycosides. Future studies will experimentally test these ideas and define the structure and activity of the *P. aeruginosa* MexXY-OprM efflux system using biochemical, computational, microbiological, structural biology approaches with the long-term goal of developing novel efflux inhibitors to counter the antibiotic resistance the systems provide to pathogenic bacteria.

2a. **Dey, D.**, *Kavanaugh, L.G.* and Conn, G.L. (2020). Antibiotic substrate selectivity of *Pseudomonas aeruginosa* MexY and MexB efflux systems is determined by a Goldilocks affinity. *Antimicrob. Agents Chemother.* **64**(8), e00496-20. [PMCID: PMC7526836]

3. EftM trimethylation of EF-Tu and its impact on *P. aeruginosa* physiology and pathology. In collaboration with Dr. Joanna Goldberg (Emory, Pediatrics) we showed that the activity of the *P. aeruginosa* methyltransferase EftM is regulated via a novel mechanism: direct thermoregulation of the protein's structure itself. EftM trimethylates lysine 5 (K5) of the translation factor EF-Tu in a manner we hypothesize mimics the phosphorylcholine modification which is important for host-cell adhesion and virulence of other opportunistic pathogens. Our first collaborative study was important because it provided a mechanistic basis for how *P. aeruginosa* may control expression of this modification (**ref. a**). Subsequent work revealed an additional contribution to regulation of EftM expression at the transcriptional level and also that EF-Tu modification K5 has no impact on its canonical role in translation, suggesting its purpose is potentially exclusive to the surface-localized role in host cell adhesion (**ref. b**). Finally, we recently completed a study revealing details of the molecular mechanism of EF-Tu recognition by EftM which appears to exploit strategies common to both Class I Rossmann fold and the eukaryotic SET domain methyltransferases (**ref. c**). This work sets the scene for future studies on EftM structure/ enzymatic mechanism (in particular the similarity of its active site to those of SET methyltransferases) that will provide a framework for future development of specific inhibitors of EF-Tu K5 trimethylation to impact *P. aeruginosa* virulence.

3a. Owings, J.P.*, *Kuiper, E.G.**, Prezioso, S.M., Meisner, J., Varga, J.J., Zelinskaya, N., Dammer, E.B., Duong, D.M., Seyfried, N.T., Albertí, S., Conn, G.L. and Goldberg, J.B. (2016). *Pseudomonas aeruginosa* EftM is a thermoregulated methyltransferase. *J. Biol. Chem.* **291**(7), 3280-90 (*Co-first authors) [PMCID: PMC4751374]

3b. Prezioso, S.M., Duong, D.M., *Kuiper, E.G.*, Deng, Q., Dammer, E.B., Seyfried, N.T., Albertí, S., Conn, G.L. and Goldberg, J.B. (2019). Methylation of elongation factor-Tu by the dual thermoregulated methyltransferase EftM does not impact its canonical function in translation. *Scientific Reports*, **9**(1):3553. [PMCID: PMC6401129]

3c. *Kuiper, E.G.**, **Dey, D.***, LaMore, P.A., Owings, J. P., Prezioso, S. M., Goldberg, J. B., and Conn, G.L. (2019). Substrate recognition by the Pseudomonas aeruginosa EF-Tu methyltransferase EftM. *J. Biol. Chem.*, **294**, 20109-20121. [PMCID: PMC6937583] (*Co-first authors)

4. Non-coding RNA structure and regulation of proteins of the human innate immune system. My lab has long-standing interests in understanding the structure and activity of viral and cellular non-coding RNAs, and their mechanisms of regulation of the host cell innate immune system double-stranded (ds)RNA-sensors, dsRNA-activated protein kinase (PKR) and oligoadenylate synthetase 1 (OAS1). Our early work defined the stabilities and roles of the conserved domains within the adenovirus non-coding RNA, VA RNA_I, and more recently centered on nc886, a *cellular* ncRNA proposed to be an endogenous regulator of PKR. These studies have defined the nc886 RNA structure and revealed that only one conformer of nc886 can adopt a (currently undefined) tertiary structure that confers both potent inhibition of PKR and activation of a second dsRNA-sensing innate immune protein, OAS1, which has become the main current focus of our work in this area. In response to dsRNA, 2',5'-oligoadenylate synthetase (OAS) proteins produce 2',5'-linked oligoadenylate second messengers for which the only known target is the latent ribonuclease, RNase L. Activation of the OAS/RNase L pathway triggers a program of cellular and viral RNA degradation designed to halt protein synthesis in the infected cell. First, we reported the discovery of a novel *single-stranded* RNA motif (termed 3'-ssPy, or "*three prime spy*") that strongly potentiates OAS1 activation by a short model dsRNA duplex as well as structured viral and cellular non-coding RNAs (**ref. a**). Subsequently, we identified the role of a novel tertiary structure within nc886 in potent the activation of the OAS/RNase L pathway both *in vitro* and in the context of cellular innate immune signaling (**ref. b**). Our most recent studies have revealed that even for "simple" model dsRNAs there is a potentially complex interplay of RNA features that controls whether OAS1 becomes activated or not (e.g. **ref. c**,**d**) and we are currently working to fully define the "rules" that govern potent OAS1 activation by dsRNA and the impacts of

motifs like 3'-ssPy.

4a. Vachon, V.K., Calderon, B.M.[¶] and Conn, G.L. (2015). A novel RNA molecular signature for activation of 2'-5' oligoadenylate synthetase-1. *Nucleic Acids Res.* **43**(1), 544-552. [PMCID: PMC4288181]

4b. Calderon, B.M.[¶] and Conn, G.L. (2018). A human cellular noncoding RNA activates the antiviral protein 2'–5'-oligoadenylate synthetase 1. *J. Biol. Chem.* ***293****, 16115-16124.* [PMCID: PMC6187638]
This article was an Editors' Pick for the October 12th, 2018 issue of *JBC*, see:
Dinman, J.D. (2018). Shapeshifting RNAs guide innate immunity. *J. Biol. Chem.* ***293****, 16125-12126.*

4c. Schwartz, S.L., Park, E.N.[#], Vachon, V.K., Danzy, S., Lowen, A.C. and Conn, G.L. (2020). Human OAS1 activation is highly dependent on both RNA sequence and context of activating RNA motifs. *Nucleic Acids Res*. **48**(13), 7520–7531. [PMCID PMC7367156]

4d. Schwartz, S.L., **Dey, D.**, Tanquary, J.[†], Bair, C.R., Lowen, A.C. and Conn, G.L. (2022) Role of helical structure and dynamics in oligoadenylate synthetase 1 (OAS1) mismatch tolerance and activation by short dsRNAs. *Proc. Natl. Acad. Sci. U. S. A.*, **119**, PMCID: PMC8784149.

5. Molecular bases of OAS IEIs. As part of a multinational interdisciplinary team, we recently reported the identification and mechanistic basis of a new IEI resulting from four distinct heterozygous mutations in the human *OAS1* gene which encodes the enzyme oligoadenylate synthetase 1 (OAS1; **ref. a**). The OAS family of proteins is responsible for sensing foreign (e.g. viral) double-stranded (ds)RNA and promoting an interferon-induced innate immune response. In humans, three catalytically active OAS proteins (OAS1-3) accomplish this by dsRNA-activated synthesis of 2',5'-linked oligoadenylate (2,5-A) second messengers which then activate the latent ribonuclease (RNase L). Our studies showed that the four OAS1 variants possess low-level gain-of-function (GoF) activity, i.e. 2,5-A synthesis in the *absence of dsRNA activation*, and thus result in a new IEI we termed OAS1-associated polymorphic auto-inflammatory immunodeficiency disorder (OPAID). Using computational modeling and molecular dynamics (MD) approaches, my group that showed each OAS1 GoF amino acid substitution results in similar changes in protein dynamics surrounding the active site, despite being distributed across one half of the protein. In our on-going work, we will next define the molecular basis for OAS1 GoF, including the mechanism of allosteric communication between the GoF sites and residues surrounding the active site. Defining these mechanisms also promises to significantly deepen our understanding of how OAS1 is regulated, both by dsRNA activators and by protein residue networks that limit aberrant activation in the absence of dsRNA. Recently, through our global IEI surveillance network we also identified a first heterozygous missense mutation in *OASL* leading to OASL gain-of-function activity and dysregulation of innate immune signaling. As noted in the NIAID Notice of Special Interest (NOSI) "*Investigations on Inborn Errors of Immunity/Primary Immunodeficiencies*" (NOT-AI-21-032), efforts to understand genetic defects leading to *even exceptionally rare IEIs* offer unparalleled potential for novel insights into the fundamental molecular and cellular mechanisms of immune system function. Our goal will therefore be to establish a foundation from which to exploit the OASL variant and its resultant IEI to define currently unappreciated interplay between innate immune sensing of DNA and RNA viruses via the OASL/ RIG-I and cGAS/ STING pathways.

5a. Magg, T., Okano, T., Koenig, L.M., Boehmer, D.F.R., Schwartz, S.L., Inoue, K., Heimall, J., Licciardi, F., Ley-Zaporozhan, J., Ferdman, R.M., Caballero-Oteyza, A., Park, E.N.[#], Calderon, B.M.[¶], **Dey, D.**, Kanegane, H., Cho, K., Montin, D., Reiter, K., Griese, M., Albert, M.H., Rohlfs, M., Gray, P., Walz, C., Conn, G.L., Sullivan, K.E., Klein, C., Morio, T. and Hauck, F. (2021) Heterozygous OAS1 gain-of-function variants cause an autoinflammatory immunodeficiency. *Science Immunology*, **6**.PMCID: PMC8392508.