BIOGRAPHICAL SKETCH

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NAME: Conn, Graeme Leslie

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

POSITION TITLE: Professor of Biochemistry

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	Structural Biology
Johns Hopkins University	Postdoc	12/1999	Structural Biology

A. Personal Statement

My role on this request for access to the NNCAT Krios microscope facility will be to collaborate with Dr. Dunham on the determination of ribosome subunit-rRNA methyltransferase complexes. 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. As such, we have an established track-record of using a broad array of approaches including biochemistry, structural and other biophysical methods, molecular biology and microbiology to dissect fundamental biological mechanisms related to this topic. We will apply this knowledge to support the determination of new, high-resolution ribosome subunit-rRNA methyltransferase complex structures using cryo-EM. Specifically, for the work proposed here, my group will prepare three complexes: 30S-RmtB, and 30S- and 50S-TlyA complexes. RmtB is a clinically relevant aminoglycoside-resistance 16S rRNA methyltransferase, while the dual substrate (30S/50S) enzyme incorporates two modifications *required* for binding and activity of drugs like capreomycin, which are essential second line treatments against drug-resistant *M. tuberculosis*. Determining these structures will represent a major advance in our understanding of how these enzymes carry out their function in relation to antibiotic resistance and, more generally, about how rRNA modification enzymes recognize and site-specifically modify the bacterial ribosome.

B. Positions and Honors

Positions and Employment

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

Other Experience, Service and Professional Memberships

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 2016).

2001-present *Ad hoc* grant reviewer/ study section: The Wellcome Trust, BBSRC (UK); 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); Canadian Council for

the Arts (Killam Research Fellowship) and the Advanced Laureate Awards (ALA) programme, Irish Research Council (IRC).

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.*

2012 'Recombinant and *in vitro* RNA synthesis: Methods and Protocols' (Editor), Methods in Molecular Biology series (Walker, J.M., series Editor), Humana Press.

2012 Co-chair, Proteins and Crystallography Committee 2, American Heart Association.

2013-2014 Chair, Proteins and Crystallography Committee 2, American Heart Association.

2014-present Frontiers in Molecular Biosciences, Reviewing Editor (Structural Biology)

2015 NIH/CSR ZRG1 F13-C 20 L, Fellowships: Infectious Diseases and Microbiology (twice).

2016 NIH/CSR ZRG1 F13-C 20 L, Fellowships: Infectious Diseases and Microbiology (twice).

2017-2019 Guest Editor, special topic "*Bacterial Mechanisms of Antibiotic Resistance: A Structural Perspective*", Frontiers in Molecular Biosciences.

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).

Awards/Honors

1990–1993 Faculty of Science and Engineering Bursary, University of Edinburgh, UK.

1990–1994 1st, 3rd and 4th Year Undergraduate Class Prize (top ranked student), Department of Chemistry, University of Edinburgh, UK.

1993–1996 Royal Society of Edinburgh Caledonian Trust Scholarship (PhD).

1996–2000 Wellcome Trust International Traveling Prize Fellowship (Postdoctoral).

2000–2004 Wellcome Trust Independent Research Career Development Fellowship.

2018 Hidden Gem award, Emory University School of Medicine.

C. Contribution to Science

Complete List of Published Work in My NCBI (67 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]

1. Molecular basis for aminoglycoside-resistance arising from 16S rRNA methylation. 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 drugs like plazomicin. For the last decade or so, we have been at the forefront of efforts to determe the structures of these enzymes and to define the molecular features which govern their interactions with cosubstrate S-adenosyl-L-methionine (SAM) and 30S substrate. In 2014 in collaboration with Dr. Dunham's group, we presented a major 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 built on by a detailed mechanistic study from my lab which dissected the molecular basis of 30S recognition by NpmA (**ref. c**). Over the course of our previous periods of funding on this award, our lab also completed structure-function studies of multiple m^1A1408 enzymes from both aminoglycoside-producing and human pathogenic bacteria deepening our understanding of the mechanisms of action of these resistance determinants (e.g. **ref. b**). 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 efforts to obtain complementary structure-function insights for members of the m^7G1405 family (**ref. d**) which represent the greater clinical threat to aminoglycoside efficacy. Our long-term goal is to exploit the understanding we develop of these enzymes and their target recognition mechanisms, as well as the nature of antibiotic-methylated rRNA interactions, to facilitate development of specific inhibitors of these resistance determinants.

a. 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]

b. Savic, M., Sunita, S., Zelinskaya, N., Desai, P.M., Macmaster, R., Vinal, K. and **Conn, G.L.** (2015). 30S subunit-dependent activation of the *S. cellulosum* So ce56 aminoglycoside-resistance 16S rRNA methyltransferase Kmr. *Antimicrob. Agents Chemother.* **59**(5), 2807-2816. [PMCID: PMC4394793]

c. Vinal, K. and **Conn, G.L.** (2017). Molecular mechanism of substrate recognition and modification by a pathogen-derived aminoglycoside-resistance 16S rRNA methyltransferase. *Antimicrob. Agents Chemother.* **61**(5), pii: e00077-17. [PMCID: PMC5404524]

d. Nosrati, M., Dey, Debayan, D. Strassler, S.E., Zelinskaya, N. and **Conn, G.L.** (2019). Critical residues in the aminoglycoside-resistance 16S rRNA (m^7G1405) methyltransferase RmtC play distinct roles in 30S substrate recognition.*J. Biol. Chem. 294, 17642-17653.* [PMCID: PMC6873201]

2. 30S and 50S ribosome subunit recognition by *M. tuberculosis* TlyA. We recently established a new direction in our studies of substrate recognition by rRNA methyltransferases focused on understanding the dual 30S (16S rRNA) and 50S (23S rRNA) substrate specificity of the 2'-O-cytidine methyltransferase TlyA from *M. tuberculosis.* TlyA incorporates 2'-O-methyl modifications on a single site within each ribosome subunit, C1409 (*E. coli* numbering) in the 16S rRNA decoding center (adjacent to the two aminoglycoside-resistance modifications at G1405 and A1408) and at C1920 in 23S rRNA (in Helix 69 which forms an intersubunit bridge near the decoding center). TlyA is a mycobacterial "housekeeping" methyltransferase and these modifications, which are thus intrinsically present in mycobacterial ribosomes, *enhance* binding of capreomycin, an antibiotic used as a second line drug in the treatment of TB. We determined the structure of the TlyA C-terminal methyltransferase domain and identified a short, functionally critical motif connecting it to the N-terminal domain which presumed to play a major role in specific substrate recognition (**ref. a**). Studies of TlyA, as have the potential to greatly expand our fundamental understanding of ribosome-methyltransferase interactions. First, on the 30S, this enzyme must recognize the same or similar structural features as the two subfamilies of aminoglycoside-resistance enzyme which will provide broad new insights into the general molecular strategies used by distinct rRNA methyltransferase, particularly those which impact antibiotic action, to recognize a common substrate. Second, these studies will also reveal how a single enzyme accomplishes the molecular feat of recognizing the same target site (for TlyA, a cytidine ribose 2'-OH) within two structurally distinct contexts (30S vs 50S).

a. Witek, M.A.*, Kuiper, E.G.*, Minten, E., Crispell, E.K. and **Conn, G.L.** (2017). A novel motif for S-adenosyl-L-methionine binding by the ribosomal RNA methyltransferase TlyA from *Mycobacterium tuberculosis*. *J. Biol. Chem.* **292**(5), 1977-1987. (*Co-first authors) [PMCID: PMC5290967]

3. EftM trimethylation of EF-Tu and its impact on *P. aeruginosa* physiology and pathology. We recently established a collaboration with Joanna Goldberg's lab (Emory, Department of Pediatrics) to show that the activity of the *P. aeruginosa* EF-Tu methyltransferase (EftM) is dual thermoregulated enzyme with part of this regulation arising via a novel mechanism: direct thermoregulation of the protein's structure itself (**ref. a,b**). EftM trimethylates lysine 5 (K5) of the translation factor EF-Tu in a manner we hypothesize mimics the phosphorylcholine modification important for hold-cell adhesion and virulence of other opportunistic pathogens These first collaborative studies were important because they provided a mechanistic basis for how *P. aeruginosa* may control expression of this modification on its surface and also set the scene for on-going studies of EftM methyltransferase structure, and mechanisms of specific EF-Tu recognition and modification (**ref. c**). Such studies will provide a framework for future development of specific inhibitors of EF-Tu K5 trimethylation to impact *P. aeruginosa* virulence.

a. 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]

b. 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. *Sci. Rep.* **9**(1):3553. [PMCID-PMC6401129]

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

4. Non-coding RNA structure and regulation of the innate immune protein PKR. My lab has long-standing interests in the structure and activity of viral non-coding RNAs, such as Adenovirus VA RNA_I – an essential, pro-viral RNA best known for inhibition of the double-stranded (ds)RNA-activated protein kinase (PKR). Our early work defined the stabilities and roles of the conserved domains within VA RNA_I (e.g. **ref a**), including the remarkable finding that the entire Terminal Stem could be deleted without loss of activity (whereas smaller deletions were detrimental). Our finding complemented the discovery that VA RNA_I is similarly processed in the cell by Dicer, offering the prospect (still to be fully explored) that Adenovirus may exploit Dicer activity to tune the activity of VA RNA_I appropriately to the stage of viral replication. Our subsequent work defined the minimal requirements for PKR inhibition by VA RNA_I, offering a satisfying explanation for why VA RNAs from different serotypes are equally effective despite their wide variation in sequence and length (**ref. b**) and also showed that both the N-terminal dsRNA binding domain and the C-terminal kinase domain of human PKR, but not its interdomain linker, contain important determinants for inhibition by viral non-coding RNAs (**ref. c**). Most recently, a graduate student in my lab, Brenda Calderon, extended this work to define the structure and activity of non-coding RNA 886 (nc886), a *cellular* ncRNA proposed to be an endogenous regulator of PKR (**ref. d)**. 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, oligoadenylate synthetase 1 (OAS1; also see Contribution #5, below, which is our current focus in the area of innate immune regulation by RNA).

a. Wahid, A.M., Coventry, V.K. and **Conn, G.L.** (2008). Systematic deletion of the adenovirus-associated RNA_I terminal stem reveals a surprisingly active RNA inhibitor of double-stranded RNA-activated protein kinase. *J. Biol. Chem.* **283**(25), 17485–17493. [PMCID: PMC2427366]

b. Wilson*, J.L, Vachon*, V.K., Sunita, S., Schwartz, S.L. and **Conn, G.L.** (2014). Dissection of the adenoviral VA RNA_I Central Domain structure reveals minimal requirements for RNA-mediated inhibition of PKR. *J. Biol. Chem*. **289**(33), 23233-23245. (*Co-first authors) [PMCID: PMC4132820]

c. Sunita S.*, Schwartz, S.L.*, and **Conn, G.L.** (2015) The Regulatory and kinase domains but not the Interdomain linker determine human double-stranded RNA-activated kinase (PKR) sensitivity to inhibition by viral non-coding RNAs. *J. Biol. Chem*. **290**(47):28156-28165. (*Co-first authors) [PMCID: PMC4653674]

d. Calderon, B.M. and **Conn, G.L.** (2017). Human non-coding RNA 886 (nc886) adopts two structurally distinct conformers that are functionally opposing regulators of PKR. *RNA RNA*, **23**(4):557-566. [PMCID: PMC5340918]

5. Molecular mechanisms of RNA-mediated regulation of OAS1. 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. 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**). Our current studies have revealed that even for "simple" model dsRNAs there is a potentially complex interplay of RNA features that controls whether OAS1 becomes activated. We next aim to extend these initial finding to define the "rules" that govern potent OAS1 activation by dsRNA and the impacts of motifs like 3'-ssPy or, as we recently reported, a novel tertiary structure within the cellular non-coding RNA 886 (nc886) on the activation of the OAS/RNase L pathway both *in vitro* and in the context of cellular infection (**ref. b**). In unpublished work, we have recently cloned and expressed human OAS3 in *E. coli* and will extend our studies to this additional component of the OAS/ RNase L pathway. For example, we will test whether similar rules about OAS-activating RNA features apply to this RNA or if OAS3 has evolved as a more general sensor longer dsRNAs. Finally, we have also recently reviewed current knowledge in the field of OAS protein regulation by RNA (**ref. c**).

a. 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]

b. Calderon, B.M. and **Conn, G.L.** A human cellular noncoding RNA activates the antiviral protein 2'–5'-oligoadenylate synthetase 1 (2018). *J. Biol. Chem. **293**, 16115-16124.* **[Editors' Pick for October 12th, 2018 issue of *JBC*]**

c. Schwartz, S.L. and **Conn, G.L.** (2019). RNA regulation of the antiviral protein 2'-5'-oligoadenylate synthetase (OAS). *WIREs RNA*, e1534. [PMCID: PMC6585406]

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AI088025 (PI–Conn; CoI–Dunham, Comstock) 05/01/2010 – 04/30/2020
NIH/NIAID
RNA modification and antibiotic resistance
This project will investigate 16S ribosomal RNA methyltransferase enzymes that confer resistance to aminoglycoside antibiotics. The goals are to determine methyltransferase and methyltransferase-30S substrate complex X-ray crystal structures and to define the molecular mechanisms which underpin target (30S ribosome) recognition by these resistance enzymes (see contribution #1, in section C, above). *Renewal application R01 AI088025 (years 11-15) scored at 12% (NIAID payline is 12%) and is pending council review.*

R01 GM130135 (MPI–Jackman*, Conn; CoI–Comstock) 08/01/2018 – 07/31/2022
NIH/NIGMS
Mechanisms and biological functions of SPOUT methyltransferases.
My group's role in this project is to define the molecular basis of specific substrate selection and modification by the m^1G9 tRNA methyltransferase Trm10 using structural, biophysical and biochemical approaches.

R01-AI144067-01(PI–Conn; CoI–Lowen) 03/12/2019 – 02/28/2023
NIH/NIAID
dsRNA regulation of the cytosolic innate immune system
This project aims to define the "rules" that govern OAS1 activation by specific dsRNA sequences and the impacts of motifs like 3'-ssPy and the novel tertiary structure within the cellular non-coding RNA 886 (nc886) on the activation of the OAS/RNase L pathway both *in vitro* and in the context of influenza infection (see contribution #5, in section C, above).

Artificial Intelligence Molecular Screens (AIMS) Award A18-106 (PI–Conn) 10/09/2018 – 09/30/2019
Atomwise
AIMS identification of small molecule inhibitors targeting the E. coli 16S rRNA methylase RmtB
Atomwise provided a 72-compound library of small molecules identified using their proprietary AI/ machine learning algorithms to bind RmtB as potential leads for inhibitor development. Analysis of compound activity is currently on-going in our lab
[Related to contribution #1, in section C, above.]

Artificial Intelligence Molecular Screens (AIMS) Award A19-030 (PI–Conn) 10/09/2019 – 09/30/2020
Atomwise
AIMS for inhibitors of human oligoadenylate synthetase 1 (OAS1)
Atomwise will provide a 72-compound library of small molecules identified using proprietary AI/ machine learning algorithms to potential OAS1 as potential leads for inhibitor development.
[Related to contribution #5, in section C, above.]

Mentored Awards

NIH/NIAID (F31-AI133950), PI-Samantha Schwartz 07/01/2017 – 06/30/2020
Regulation of 2'-5'-oligoadenylate synthetase 1 (OAS1) by dsRNA
[Related to contribution #5, in section C, above.]

Cystic Fibrosis Foundation Postdoctoral Fellowship, PI-Debayan Dey 04/01/2018 – 03/30/2020
Regulation of EF-Tu lysine trimethylation by P. aeruginosa EftM
[Related to contribution #3, in section C, above.]

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: Christine M. Dunham

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Barnard College, Columbia University, New York, NY	B.A.	05/1997	Biochemistry
University of California, Santa Cruz, CA	Ph.D.	06/2003	Structural Biology
MRC Laboratory of Molecular Biology, Cambridge, England	Postdoc	04/2008	Structural Biology

A. Personal Statement

My lab studies the regulation of gene expression at the molecular level using structural biology approaches. My role in this proposal is to collaborate with the Conn lab to solve structures of the bacterial ribosome undergoing modification in response to aminoglycoside exposure. I have been collaborating with the Conn lab for the past 10 years.

As a graduate student in Prof. William G. Scott's lab at the University of California, Santa Cruz, I used time-resolved X-ray crystallography to understand the mechanism of an RNA enzyme involved in viral rolling circle replication cycle (**a**). As an American Cancer Society Postdoctoral Fellow in Dr. Venki Ramakrishnan's lab at the MRC Laboratory of Molecular Biology in Cambridge, England, I again tackled questions of RNA function but, this time, in the context of the bacterial ribosome. Using X-ray crystallography, I solved the first high resolution structure of a bacterial ribosome containing tRNA and mRNA ligands (**b**) that provided the ability to ask important biological questions of how elongation factors function (see **Contribution 1**, **a-d**). In my own lab, we focus on mechanisms of ribosome regulation, how stress alters translation and the roles of toxin-antitoxin pairs in inhibiting protein synthesis to alter bacterial physiology. We use interdisciplinary approaches including molecular biology, biochemistry, X-ray crystallography and single particle cryo-electron microscopy (cryo-EM). We have recently determined the molecular basis for tRNA-mediated ribosomal frameshifting (**c**) and using cryo-electron microscopy (cryo-EM), we have determined how structured mRNAs control translation in a manner important for mRNA frame maintenance and co-translational folding (**d**).

a. **Dunham CM**, Murray JB, and Scott WG. (2003) A Helical Twist-Induced Conformational Switch Activates Cleavage in the Hammerhead Ribozyme. *Journal of Molecular Biology* **332**(2):327-36. PMID: 12948485.

b. Selmer M*, **Dunham CM***, Murphy IV FV, Weixlbaumer A, Petry S, Kelley AC, Weir J, and Ramakrishnan V. (2006) Structure of the 70S Ribosome Complexed with mRNA and tRNA. *Science* 313(5795):1935-42. PMID: 16959973. *These authors contributed equally.

c. Hong S*, Sunita S*, Dunkle JA, Maehigashi T and **Dunham CM**. (2018) Mechanism of +1 tRNA-mediated frameshifting. *Proc Natl Acad Sci* 115(44):11226-31. PMCID: PMC6217423. *These authors contributed equally. *This paper won the 2018 Cozzarelli Prize from the National Academy of Sciences for the Best Biological Science paper published in PNAS.*

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.

d. Zhang Y*, Hong S*, Ruangprasert A, Skiniotis Y and **Dunham CM**. (2018) Alternative modes of E-site tRNA binding in the presence of structured mRNAs at the mRNA entrance channel. *Structure.* 26(3):437-445. PMCID: PMC5842130. *These authors contributed equally.

B. Positions and Honors

Positions and Employment

1994 - 1995	NSF Summer Undergraduate Research Fellow, Albany Medical College, Albany, New York. Advisor: Professor Peter Weber.
1996	NSF Summer Undergraduate Research Fellow, University of Texas Medical Branch at Galveston. Advisor: Professor Bennett Van Houten.
2004	Medical Research Council Career Development Fellow, MRC Laboratory of Molecular Biology, Cambridge, UK. Advisor: Group Leader Venki Ramakrishnan.
2004 - 2008	American Cancer Society Postdoctoral Fellow, MRC Laboratory of Molecular Biology, Cambridge, UK. Advisor: Group Leader Venki Ramakrishnan.
2008 - 2016	Assistant Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.
2017 - present	Associate Professor, Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia.

Other Experience, Service and Professional Memberships

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).
2008 - present	Temporary grant reviewer/study section: NSF, Genes and Genome Systems (2009,2010); NIH ZRG1 Biological Chemistry and Macromolecular Physics (P01; 2010); NIH K99 Pathways to Independence Awards study section (2011); NIH Macromolecular Structure and Function C (MSFC) grant study section (2010,2018); American Heart Association, Basic Cell Protein and Crystallography grant study section (2010,2011,2012); NSF, Division of Molecular and Cellular Biosciences, Gene Expression study section (2010,2013,2014,2015, 2017,2018); NSF Division of Molecular and Cellular Biosciences, Gene Expression study section, CAREER award fellowships (2014); NIH Molecular Genetics A (MGA) grant study section (2011,2012 (twice),2014); American Cancer Society, RNA Mechanisms of Cancer grant study section (2012); NSF Graduate Student Research Fellowship predoctoral study section, Division of Molecular and Cellular Biosciences (2018); Swiss National Science Foundation grant reviewer (2015); NIH NIGMS Special Emphasis Panel, Support of Competitive Research (SCORE; 2016); Grant reviewer, Boehringer Ingelheim PhD Fonds, Germany (2016); NIH NIEHS site review, Durham, NC (2018)
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.*
2009	Session chair, “Ribosome Regulation: Assembly, Modification and Function”, ASM conference, Philadelphia, PA.
2011	Conference organizing committee, Suddath symposium on the Ribosome, Institute for Bioengineering & Bioscience, Georgia Tech, Atlanta, GA.
2012	Session chair, “Supramolecular Assemblies”, American Crystallographic Association conference, Honolulu, HI.
2013	Pew Charitable Trusts 2014 Conference organization committee, Chile.
2015	2016 Conference Organizing committee, ASBMB, San Diego, CA.
2015	Session chair, “Translation and sRNA function”, Molecular Genetics of Bacteria and Phages Meeting, Madison, WI.
2016	Session chair, “Words from the Beamline”, SER-CAT Annual Meeting, Emory University, Atlanta, GA.
2016	Session chair, “Building Molecular Machinery”, American Society for Biochemistry and Molecular Biology, San Diego, CA.
2016	Faculty mentor, GRC Microbial Stress Responses, Mt Holyoke, MA.
2018 - 2022	NIH Permanent Study Section Member, Molecular Genetics A

2018 - present Editorial Board Member, *Molecular Microbiology*
2018 - present Editorial Board Member, *Journal of Biological Chemistry*
2019 Session chair, "Structure of toxin-antitoxins", EMBO toxin-antitoxin conference, Windsor, UK.

Awards/Honors

1999 - 2003 NSF-GAANN Graduate Research Fellowship
2003 Best Poster Prize, Gordon Research Conference on Nucleic Acids (Ph.D.)
2010 - 2015 NSF Early Career Development (CAREER) Award
2011 - 2015 Pew Scholar in the Biomedical Sciences
2016 - 2021 Burroughs Wellcome Investigator in the Pathogenesis of Infectious Diseases
2017 American Crystallographic Association Etter Early Career Awardee
2018 American Society of Biochemistry and Molecular Biology (ASBMB) Young Investigator
2018 Cozzarelli Prize from the National Academy of Sciences for the Best Biological Science paper published in *PNAS*.

C. Contributions to Science

Link to a more complete list of publications (currently 33 research papers and 4 reviews/News & Views): http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/45371674/?sort=date&direction=ascending

Since I was a postdoctoral fellow in Dr. Venki Ramakrishnan's lab, I have focused my research on understanding the molecular basis of protein synthesis (**Contribution 1**). These structural insights changed the way we could mechanistically dissect translation to understand function and dysregulation. We next studied how the ribosome prevented non-canonical mechanisms of gene expression including mRNA frameshifting (**Contribution 2**). We discovered how tRNA modifications control the mRNA frame and how their absence causes allosteric dysregulation of the ribosome. Our interest in protein synthesis led us to study bacterial toxins that control translation to limit growth and cause tolerance to antibiotics (**Contribution 3**). Related to inhibition of translation, toxin biology is control by toxin suppression by antitoxins, transcriptional autorepression to limit expression, and activation by controlled proteolysis of antitoxins (**Contribution 4**). Lastly, we augment our studies with interdisciplinary collaborations to understand the regulation of protein synthesis with other research groups including the Fredrick, Conn and Skiniotis labs (**Contribution 5**).

*These authors contributed equally. #Co-corresponding authors.

1. Protein synthesis is carried out by the ribosome and is one of the most conserved biological processes. As a postdoctoral fellow in 2009 Chemistry Nobel Laureate 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 >900 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, and Ramakrishnan V. (2006) Structure of the 70S Ribosome Complexed with mRNA and tRNA. *Science* 313(5795):1935-42. PMID: 16959973.
   b. Weixlbaumer A, Petry S*, **Dunham CM***, Selmer M*, Kelley AC and Ramakrishnan V. (2007) Crystal structure of the ribosome recycling factor bound to the ribosome. *Nat Struct Mol Biol* 14(8):733-7. PMID: 17660830.
   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 and Brodersen DE. (2009) The structural basis for mRNA recognition and cleavage by the ribosome-dependent endonuclease RelE. *Cell* 139(6):1084-1095. PMCID: PMC2807027.
2. Ribosomal frameshifting is a key regulatory mechanism to control gene expression whereby the noncanonical reading of the genetic code facilitates expression of different protein products. Frameshift-prone tRNAs and mRNAs that contain complex tertiary structures to physically block unwinding by the ribosome during elongation are two major causes for the change in the mRNA reading frame. We have solved high-resolution structures 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 and **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.
b. Fagan CE, Maehigashi T, Dunkle JA, Miles SJ and **Dunham CM**. (2014) Structural insights into translational recoding by suppressor $tRNA^{SufJ}$. *RNA* 12:1944-55. PMCID: PMC4238358.
c. Hong S*, Sunita S*, Dunkle JA, Maehigashi T and **Dunham CM**. (2018) Mechanism of +1 tRNA-mediated frameshifting. *Proc Natl Acad Sci* 115(44):11226-31. PMCID: PMC6217423.
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. *This paper won the 2018 Cozzarelli Prize from the National Academy of Sciences for the Best Biological Science paper published in PNAS.*
d. Nguyen HA, Hoffer ED and **Dunham CM**. (2019) Importance of tRNA anticodon loop modification and a conserved, noncanonical anticodon stem pairing in $tRNA^{Pro}$-CGG for decoding. *J Biol Chem* 294(14):5281-91. PMCID: PMC6462517. *Selected as the Editor's Pick, an honor bestowed on the top 2% of papers published in JBC.*

3. 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. A majority of 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**). Further, we identified the small ribosomal 30S subunit as a HigB toxin target suggesting that toxins recognize the initiation phase of translation (**c**) and demonstrated which HigB residues are critical for mRNA cleavage (**d**). Our results have provided significant insights into the molecular mechanism of toxin-mediated regulation of gene expression during stress and suggest that each toxin may be tuned to a specific stress.
a. Maehigashi T*, Ruangprasert A*, Miles SJ and **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.
b. Schureck MA, Dunkle JA, Maehigashi T, Miles SJ and **Dunham CM**. (2015) Defining the mRNA recognition signature of a bacterial protein toxin. *Proc Natl Acad Sci* 112(45):13862-7. PMCID: PMC4653167.
c. Schureck MA, Maehigashi T, Miles SJ, Marquez J and **Dunham CM**. (2016) mRNA bound to the 30S subunit is a HigB endonuclease substrate. *RNA* 22(8):1261-70. PMCID: PMC4931118.
d. Schureck MA, Repack A, Miles SJ, Marquez J and **Dunham CM** (2016) Mechanism of endonuclease cleavage by the HigB toxin. *Nucleic Acids Res* 44(16):7944-53. PMCID: PMC5027501.

4. To determine the critical molecular interactions between antitoxins and toxins that contribute to toxin inactivation, we solved X-ray crystal structures of two toxin-antitoxin family members regulated by diverse stresses: *P. vulgaris* HigBA complex (**a**) and *E. coli* DinJ-YafQ complex (**b**). To understand *Mycobacterium tuberculosis* toxins involved in ribosome inhibition, we studied the structure and function of the MazF-mt6 toxin where we identified determinants for the evolutionary degeneracy of the MazF toxin family (**c**). Lastly, we identified how the *E. coli* DinJ antitoxin undergoes selectively proteolysis by Lon protease during stress to release the YafQ toxin (**d**).
a. Schureck MA, Maehigashi T, Miles SJ, Marquez J, Ei Cho S, Erdman R and **Dunham CM**. (2014) Structure of the *P. vulgaris* HigB-$(HigA)_2$-HigB toxin-antitoxin complex. *J Biol Chem* 289(2):1060-70. PMCID: PMC3887174.
b. Ruangprasert A*, Maehigashi T*, Miles SJ, Giridharan N, Liu JX and **Dunham CM**. (2014) Mechanisms of toxin inhibition and transcriptional repression by *E. coli* DinJ-YafQ. *J Biol Chem* 289(30):20559-69. PMCID: PMC4110269.
c. Hoffer EA, Miles SJ and **Dunham CM**. (2017) The structure and function of *Mycobacterium tuberculosis* MazF-mt6 provides insights into conserved features of MazF endonucleases. *J Biol Chem* 292(19):7718-26. PMCID: PMC5427253. *Cover image*
d. Ruangprasert A, Maehigashi T, Miles SJ and **Dunham CM**. (2017) Importance of the *E. coli* DinJ antitoxin carboxy terminus for toxin suppression and regulated proteolysis. *Mol Micro* 104(1):65-77.

PMID: 28164393.

5. Natural collaborations with groups having overlapping interests also resulted in significant advances in our understanding of how translation is regulated. In collaboration with the Fredrick lab, we determined the structural basis for 16S ribosomal RNA *ribosome ambiguity mutations* (*ram*) mutations (**a,b**). 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. 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 (**c**). In collaboration with the Skiniotis lab, we solved high resolution cryo-EM structures of the ribosome translating a structured mRNA that causes frameshifting (**d**).
   a. Fagan CE, Dunkle JA, Maehigashi T, Dang MN, Deveraj A, Miles SJ, Qin D, Fredrick K and **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. Hoffer ED, Maehigashi T, Fredrick K, and **Dunham CM**. (2018) Ribosomal ambiguity (*ram)* mutations promote 30S domain closure and thereby increase miscoding. *Nucleic Acids Res.* 47(3):1557-63. PMCID in progress. *Cover image.*
   c. Dunkle JA, Vinnal K, Desai PM, Zelinskaya N, Savic M, West DM, Conn GL* and **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.
   d. Zhang Y*, Hong S*, Ruangprasert A, Skiniotis G and **Dunham CM**. (2018) Alternative modes of E-site tRNA binding in the presence of structured mRNAs at the mRNA entrance channel. *Structure.* 26(3):437-445. PMCID: PMC5842130.

D. Research Support

Ongoing Research Support

R01 GM093278 Dunham (PI) 09/01/19-08/31/23
NIH/NIGMS
Molecular basis of ribosomal frameshifting. This project aims to understand the molecular and biochemical basis for bacterial ribosomal frameshifting resulting from modification deficient tRNAs or complex mRNAs.

Cystic Fibrosis Foundation New Investigator, DUNHAM19I0 Dunham (PI) 10/01/9- 09/30/21
Visualizing Co-translational Folding of CFTR. This project aims to determine the molecular basis of CFTR Δ508 folding defects on the ribosome.

Investigator in the Pathogenesis of Infectious Diseases
Burroughs Wellcome Fund Dunham (PI) 07/01/16-06/30/21
Characterization of Pathways involved in Bacterial Persistence and Antibiotic Resistance**.** This project aims to determine the molecular mechanisms by which bacteria activate toxins in response to stress.

NSF CHE 1808711 Dunham (PI) 08/01/18-07/31/21
Expanding the genetic code: the rationale design of frameshift suppressor tRNAs in recoding. This project aims to expand the coding capacity of tRNAs using a rational, structure-based redesign.

R01 GM065183 Ibba, Kearns, Dunham (MPI) 09/01/17- 08/31/21
NIH/NIGMS
Mechanisms of Translational Control. This project aims to understand the mechanism of ribosome stalling during poly-proline stretches.

R01 AI088025 Conn (PI) 05/01/15- 04/30/20
NIH/NIAID
RNA modification and antibiotic resistance**.** This project investigates how ribosomal RNA methyltransferase enzymes confer resistance to aminoglycoside antibiotics.

R01 GM121650-01A1 Keiler (PI) Penn State 08/01/17-07/30/21
Ribosome Rescue. This project focuses on understanding why ribosome rescue pathways inhibit bacterial growth. Role: subcontract