BIOGRAPHICAL SKETCH

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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 lead my group in the solving of ribosome structures.

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