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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Barnard College, Columbia University, New York, NY	B.A.	05/1997	Chemistry
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 recent 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.My role in this BAG proposal is to solve structures of stalled ribosomes bound to a novel rescue factor and drugs, to solve a structure of a ribosome undergoing mRNA frameshifting and how translation factors can influence such a state, and to solve structures of the ribosome undergoing modification in response to aminoglycoside exposure (BAG Sub Project 1).

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 the 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, 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 (section 1, **a-d**). In my own lab, we study the molecular basis of ribosome regulation and dysregulation using biochemical, X-ray crystallographic and, more recently, single particle cryo electron microscopy (cryoEM) approaches. 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 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.
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 (two times); NIH K99 Pathways to Independence Awards study section (once); NIH Macromolecular Structure and Function C (MSFC) grant study section (once); American Heart Association, Basic Cell Protein and Crystallography grant study section (three times); NSF, Division of Molecular and Cellular Biosciences, Gene Expression study section (three times); NSF Division of Molecular and Cellular Biosciences, Gene Expression study section, CAREER award fellowships (once); NIH ZRG1 Biological Chemistry and Macromolecular Physics (P01; one time); NIH Molecular Genetics A (MGA) grant study section (three times); American Cancer Society, RNA Mechanisms of Cancer grant study section (once); NSF Graduate Student Research Fellowship predoctoral study section, Division of Molecular and Cellular Biosciences (once); Swiss National Science Foundation grant reviewer (once).
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.
2020 Session chair, “Ribosomes”, CSHL Translational Control Meeting.

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, National Academy of Sciences, Best Biological Sciences paper in *PNAS*

C. Contribution to Science

Link to a more complete list of publications (currently 33 research papers and 4 reviews/book chapters): 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 interests 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. As a postdoctoral fellow in Venki Ramakrishnan's lab, I solved the first x-ray crystal structure of a frameshift suppressor tRNA bound to the 30S decoding center (**a**). These studies provided an alternative model for how tRNAs facilitate a change in the reading frame. In my own lab, I 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 (**b,c**). Further, we discovered how tRNA modifications maintain the mRNA frame and how dysregulation results in the ribosome losing its grip on the mRNA (**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 in progress.

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. 294(14):5281-91. PMCID: PMC6462517. *Selected as the Editor's Pick, an honor bestowed on the top 2% of JBC papers.*

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 address what are the critical molecular interactions between antitoxin and toxin that inhibit toxin activity, 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, epub Nov 22.* 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, NIH/NIGMS Dunham (PI) 09/01/19-08/31/23
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.

R01 AI088025, NIH/NIAID Conn, Dunham (MPI) 05/01/20- 04/30/25
*RNA modification and antibiotic resistance***.** This project investigates how ribosomal RNA methyltransferase enzymes confer resistance to aminoglycoside antibiotics.

Cystic Fibrosis Foundation New Investigator, DUNHAM19I0 Dunham (PI) 11/01/19- 10/31/21
Visualizing Co-translational Folding of CFTR. This project aims to determine the molecular basis of CFTR ∆508 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, NIH/NIGMS Ibba, Kearns, Dunham (MPI) 09/01/17- 08/31/21
Mechanisms of Translational Control. This project aims to understand the mechanism of ribosome stalling during poly-proline stretches.

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

Carb-X (Combating Antibiotic Resistant Bacteria) Microbiotix (PI) 11/01/19-10/31/21
EF-Tu binding acylaminoheterocycles targeting MDR Neisseria Gonorrhoeae. The role of the Dunham lab is to solve high-resolution structures of EF-Tu bound to antibiotics. Role: Consortium Co-Investigator

NSF CHE 2003157 Weinert (PI) 08/01/20-07/30/23
Collaborative Research: Heme Distortion and Protein-Protein Contacts in Oxygen-Dependent Globin Coupled Sensor Signaling Project. The role of the Dunham lab is to solve high-resolution structures of globin coupled sensors. Role: subcontract

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 BAG access to the NCCAT Krios microscope facility will be to serve as one of the co-applicants and sub-project leaders along with Drs. Dunham and Ghalei. The specific areas my lab will focus on are the determination of ribosome subunit-rRNA methyltransferase complexes and methylated ribosome-drug complexes (sub-projects 2a and 2b). 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 (e.g. **ref. d**) 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. 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.

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

Ongoing Research Support

R01 AI088025 (PI–Conn; CoI–Dunham, Comstock) 05/01/2010 – 04/30/2025
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).

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: Homa Ghalei, Ph.D.

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

POSITION TITLE: Assistant 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
Tehran University, Tehran, Iran	B.Sc.	05/2005	Cellular and Molecular Biology
Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany	M.Sc.	03/2007	Molecular and Structural Biology
Max-Planck Institute for Biophysical Chemistry, Göettingen, Germany	Ph.D.	11/2010	Structural Biochemistry
Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany/ Freie Universität Berlin, Berlin, Germany	Postdoctoral Fellow	03/2012	Structural Biochemistry
The Scripps Research Institute, Florida, USA	Research Associate	10/2017	Integrative Structural and Computational Biology

A. Personal Statement

I am delighted for the opportunity to participate in this Block Allocation Group (BAG) application for time access at NCCAT. Research in my laboratory focuses on understanding how the ribosome is assembled and how spatial and temporal dysregulation of ribosomal RNA modifications affects gene expression. Ribosomal RNA modifications are controlled by noncoding RNAs that are dysregulated in several human diseases including cancers. The molecular link between the levels of these ncRNAs and disease states is largely unexplored. My team examines the mechanisms that underpin aberrant ribosome production and translation caused by rRNA modification changes in biology and disease (see **contribution 4** in section C). In my training and in my own lab, I have taken the strategy of coupling in vitro biochemical and structural biology approaches with in vivo genetic studies to understand different aspects of RNA-protein complex assembly that impact gene expression regulation. As a fellow of the International Max Planck Research School (IMPRS) for molecular biology, in the laboratories of Drs. Markus Wahl and Reinhard Lührmann, I used X-ray crystallography, cryo-EM, and biochemical assays to understand the mechanism of assembly of small nuclear and nucleolar ribonucleoprotein complexes (**contribution 1**). During my postdoctoral studies in Dr. Katrin Karbstein's lab at Scripps Research Institute, I acquired skills for analyses of ribosome assembly intermediates. As a postdoc, I described novel quality control checkpoints required for cytoplasmic maturation of small ribosomal subunits and revealed the mechanism of action of several assembly factors involved in this process. My research interest in the area of ribosome biogenesis has resulted in significant contributions to understanding the molecular mechanisms that contribute to gene expression regulation and allow cells to generate diverse ribosome populations for promoting their survival under stress (**contributions 2 & 3**).

B. Positions and Honors

Positions and Employment

09/2005 – 11/2010 Fellow of the International Max Planck Research School (IMPRS) for Molecular Biology, Göettingen, Germany.

11/2010 – 03/2012	DRS Postdoctoral Research Fellow – Department of Structural Biochemistry, Freie Universität Berlin, Berlin, Germany/ Department of Cellular Biochemistry, Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany.
04/2012 – 10/2017	Research Associate – Department of Cancer Biology/Department of Integrative Structural and Computational Biology, The Scripps Research Institute, Florida, USA.
10/2017 – Present	Assistant Professor – Department of Biochemistry, Emory University School of Medicine, Georgia, USA.

Selected Other Experience and Professional Memberships

2017 – Present	Member: RNA Society (since 2008), American Society for Biochemistry and Molecular Biology (ASBMB, since 2017)
2017 – Present	Manuscript reviewer: BMC Molecular Biology, PLOS ONE, RNA Biology, BBA - Gene Regulatory Mechanisms
2017 – Present	Co-director, Biochemistry Departmental Seminar Series, Emory University School of Medicine
2018	Faculty mentor, Mentoring Luncheon, 23rd RNA Society Meeting, University of California Berkeley
2018	RNA Society Spotlight Writing Committee
2018 – Present	Mentor, Biochemistry, Cell, and Developmental Biology Training Program
2018 – Present	Member, Genetics and Molecular Biology Graduate Program
2019	Session chair, "RNA and Gene Expression session", Southeast yeast regional meeting (SERYM), Atlanta
2019 – Present	Co-organizer, G•A RNA Salon (funded by RNA Society)
2020 – Present	Mentor, Emory IRACDA Postdoctoral training program (Fellowships in Research and Science Teaching (FIRST))
2020 – Present	Mentor, NIH T32 Training Grant Biochemistry, Cell and Developmental Biology Program
2020 – Present	Executive Committee member and Director of Student Progress, Biochemistry, Cell and Developmental Biology program
2020	Temporary grant reviewer, NIH Cancer Molecular Pathobiology study section

Honors

06/2005	University of Tehran Award, *First Rank* in Graduates of Cellular and Molecular Biology
09/2005	Master Award, Faculty of Science, University of Tehran
09/2005 – 03/2007	International Max Planck Research School (IMPRS) Scholarship
04/2007 – 04/2008	Max Planck Research Society Fellowship
04/2008 – 10/2010	International Max Planck Research School (IMPRS) Fellowship
10/2010	*summa cum laude* Award for PhD Thesis
11/2010 – 03/2012	Dahlem Research School (DRS) Postdoctoral Fellowship
07/2013	Best poster prize, Ribosome meeting, Napa Valley, CA, USA
04/2012 – 09/2017	Women's Cancer Awareness Days (WCAD) Fellowship

C. Contributions to Science

1. Mechanism of assembly of structurally and compositionally related sn(o)RNPs. One of the fundamental questions in biology is how compositionally similar and structurally related complexes are distinguished in the cell and recruited for different purposes. During my graduate studies, I used biochemical analyses, X-ray crystallography, cryo-EM (in collaboration with Dr. Holger Stark) and mass spectrometry (in collaboration with Dr. Henning Urlaub) to compare the related U4 and U4atac small nuclear ribonucleoprotein complexes (snRNPs) of the major and minor spliceosome, with the box C/D small nucleolar RNPs (snoRNPs) involved in chemical modification of ribosomal (r)RNAs. By performing a thorough structural and biochemical analyses, I described the molecular determinants responsible for the differential stability of these complexes and the mechanism by which they distinguish their binding partners (**a,b**). As a graduate student, I also studied the role of Rbp18, a protein of unknown structure, which was originally identified as a candidate assembly factor for archaeal box C/D sRNPs. I determined the crystal structures of two Rbp18 proteins (from two sub-families), de novo, and uncovered a surprising diversity in their structures providing novel modes of oligomerization. By using various

functional assays, I revealed that contrary to the published data this family of proteins is single-stranded DNA binding rather than RNA binding factors and does not have any effect on box C/D s(no)RNP assembly (**c**).

a. **Ghalei H**, He-Hsuan H, Urlaub H, Wahl MC and Watkins NJ. (2010) A novel Nop5-sRNA interaction that is required for efficient archaeal box C/D sRNP formation. ***RNA*** 16(12): 2341-8. PMID: 20962039

b. Liu S*, **Ghalei H***, Lührmann R and Wahl MC. (2011) Structural basis for the dual U4 and U4atac snRNA-binding specificity of spliceosomal protein hPrp31. ***RNA*** 17(9): 1655-63. PMID: 21784869 (*equal contribution)

c. **Ghalei H***, von Moeller H*, Eppers D, Sohmen D, Wilson DN, Loll B, Wahl MC. (2014) Entrapment of DNA in an intersubunit tunnel system of a single-stranded DNA-binding protein. ***Nucleic Acids Research*** 42(10): 6698-708. PMID: 24744237 (*equal contribution)

2. Quality control checkpoints in cytoplasmic maturation of small ribosomal subunits. During my postdoctoral training, I discovered novel quality control steps that regulate the assembly and maturation of small ribosomal subunits (40S). I revealed that the essential function of the casein kinase Hrr25 (CK1δ in humans) is in ribosome assembly, where it regulates the late steps of 40S maturation and allows the pre-ribosomes to enter a quality control cycle for final maturation or degradation. This work also validated the ribosome biogenesis pathway as a potential therapeutic target in cancer (**a**). In another related project, I revealed an important quality control step which tests the ability of 40S subunits to translocate the mRNA-tRNA pair during maturation. These results showed how to progress in the maturation of 40S is linked to a functional test. My data demonstrated, for the first time, that the translation-like cycle of 40S maturation is a quality control mechanism that ensures the fidelity of the cellular ribosome pool (**b**). As a postdoc, I also uncovered the function of the ribosome biogenesis factor Ltv1 and showed that it plays a critical role in the recruitment and positioning of ribosomal proteins in the head of the small subunit (**c**). More recently, I contributed to the study of the essential ribosome biogenesis factor Rio1 which led to the discovery of another quality control checkpoint for the production of ribosomes (**d**). Together, my postdoctoral studies revealed three essential regulatory mechanisms that control the cytoplasmic production of small ribosomal subunits.

a. **Ghalei H**, Schaub FX, Doherty JR, Noguchi Y, Roush WR, Cleveland JL, Stroupe ME & Karbstein K. (2015) Hrr25/CK1δ-directed release of Ltv1 from pre-40S ribosomes is necessary for ribosome assembly and cell growth. ***Journal of Cell Biology*** 208(6):745-759. PMID: 25778921

b. **Ghalei H*,** Trepreau J*, Collins JC, Bhaskaran H, Strunk BS and Karbstein K. (2017) The Fap7 ATPase tests the ability to carry out translocation-like conformational changes to release Dim1 during 40S ribosome maturation. ***Molecular Cell*** 67(6): 990-1000. PMID: 28890337 (* equal contribution)

c. Collins JC*, **Ghalei H***, Huang H, Doherty JR, Culver RN and Karbstein K. (2018) Ribosome biogenesis factor Ltv1 chaperones the assembly of the small subunit head. ***Journal of Cell Biology*** 217(12):4141-4154. PMID: 30348748 [selected by the editors for a *Special Collection of recently published outstanding articles in JCB*] (* equal contribution)

d. Parker MD, Collins JC, Korona B, **Ghalei H** and Karbstein K. (2019) A kinase-dependent checkpoint prevents the escape of immature ribosomes into the translating pool. ***PLOS Biol*** 17(12):e3000329. PMID: 31834877

3. Mechanistic insights into the cytoplasmic assembly of small ribosomal subunits. In collaboration with the group of Dr. Elizabeth Stroupe at Florida State University, we obtained a series of structures of the immature small subunit (pre-40S) by cryo-EM, during my postdoctoral training. My role in these projects was to identify the conditions that would allow the isolation of precursor ribosomal complexes for structural analyses. I also established a partial in vitro reconstitution system that enabled us to look at otherwise unstable ribosomal assembly intermediates. Furthermore, I carried out the biochemical and in vivo yeast genetic experiments required for characterization of the isolated complexes, which allowed the unbiased positioning of the assembly factor models/structures into their corresponding electron density maps. Our biochemical and structural data suggested that maturation of the 3'-end of 18S rRNA is regulated by dissociation of the assembly factor Dim1 (**a**). More recently, I established the stalling and isolation of the 80S-like assembly intermediate that forms during late cytoplasmic maturation of 40S. This structure, which is currently under revision, revealed how the subunit interface in 80S-like intermediate is remodeled to accommodate the 60S subunit and the assembly factors Tsr1

and Dim1 (**c**). These data demonstrated how quality-control and 40S maturation are linked during ribosome assembly. More recently, we also demonstrated that the ability of the nascent 40S subunit to adopt the scanning complex is tested during assembly via structural mimicry in 80S-like complexes (**d**).

As a postdoc, I also contributed to a project describing a novel approach to separate two ribosome populations from the same cells and used this method in combination with RNA-Seq to identify mRNAs bound to yeast ribosomes with and without Rps26, a protein linked to the pathogenesis of Diamond-Blackfan anemia (DBA). My role in this project was to guide and train a graduate student to establish the purification protocol that allowed us to capture the ribosomes lacking Rps26. I also did all the quantitative compositional analyses of the purified ribosomes under different stress conditions. Our results revealed that Rps26 contributes to the mRNA-specific translation by recognition of the Kozak sequence in well-translated mRNAs and that Rps26-deficient ribosomes preferentially translate mRNAs from select stress-response pathways. These results described the novel paradigm of the production of specialized ribosomes during ribosome maturation (**b**).

a. Johnson MC*, **Ghalei H***, Doxtader KA, Karbstein K and Stroupe ME. (2017) Structural Heterogeneity in Pre-40S Ribosomes. ***Structure*** 25(2):329-340. PMID: 28111018. [*Preview* by Hartwick EW and Wimberly BT (2017) Resolving Late-Stage Intermediates of Eukaryotic Ribosome Assembly. *Structure* 25(2): 216-218. PMID: 28178457] (* equal contribution)

b. Ferretti MB, **Ghalei H**, Ward EA, Potts EL and Karbstein K. (2017) Rps26 enables mRNA-specific translation by recognition of Kozak sequence elements. ***Nature Structural & Molecular Biology*** 24(9): 700-707. PMID: 28759050

c. Rai J, Parker MD, **Ghalei H**, Johnson MC, Karbstein K and Stroupe ME. (2019) Subunit joining exposes nascent pre-40S rRNA for processing and quality control, 617910, ***bioRxiv***, doi: DOI: 10.1101/617910 **[Preprint]**

d. Huang H, **Ghalei H** and Karbstein K. (2020) Quality control of 40S ribosome head assembly ensures scanning competence. ***Journal of Cell Biology.*** *Accepted.* DOI: 10.1083/jcb.202004161.

4. Regulation of snoRNP assembly. RNA chemical modifications are prevalent in coding and non-coding RNAs. The chemical modifications of ribosomal (r)RNA play a critical role in the proper production of ribosomes that can accurately perform protein synthesis. A prominent rRNA modification is 2'-O-methylation of the ribose groups which is guided by box C/D snoRNAs. These snoRNAs interact with a set of evolutionarily conserved proteins to form snoRNPs. In eukaryotes, the formation of snoRNPs requires a set of assembly factors. Bcd1, an essential zinc finger HIT protein functionally conserved in eukaryotes, has been implicated as an early regulator for biogenesis of box C/D snoRNPs and controls steady-state levels of box C/D snoRNAs through an unknown mechanism. My lab recently used the power of yeast genetics and biochemical techniques to identify a region of Bcd1 required for two of its key functions: binding to the core protein Snu13 and recognition of the snoRNA. Further, we showed that these interactions are critical for snoRNP assembly and ribosome biogenesis. Following up on these data, we recently discovered that levels of rRNA 2'-O-methylations decrease in a site-specific manner as a result of box C/D snoRNA dysregulation, leading to a change in the binding properties of the produced ribosomes (**a**). These results are the foundation for some of my group's future work, as outlined in the research proposal.

a. Khoshnevis K, Dreggors RE, Hoffmann T, **Ghalei H#**. (2019) A conserved Bcd1 interaction essential for box C/D snoRNP biogenesis. ***Journal of Biological Chemistry*** 294(48):18360-18371. (# corresponding author)

Complete list of published work: https://www.ncbi.nlm.nih.gov/myncbi/1fE76GVIdm95b/bibliography/public/

D. Additional Information: Research Support

Ongoing Support

MIRA award (1R35GM138123-01) Role: PI 08/2020 – 07/2025 NIH (NIGMS)
"Biogenesis of macromolecular machines for post-transcriptional regulation of translation"

Completed Support

04/2012 – 09/2017 2012 Woman's Cancer Awareness Days (WCAD) Postdoctoral Fellowship