BIOGRAPHICAL SKETCH

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NAME: Peter Shen

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

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
Brigham Young University (Provo, UT)	B.S	12/2003	Biochemistry
Brigham Young University (Provo, UT)	Ph.D.	08/2011	Biochemistry
University of Utah (Salt Lake City, UT)	Postdoctoral	08/2015	Structural biology

A. Personal Statement

The major theme of my research program is to visualize the structural landscapes of dynamic molecular machines isolated directly from their native source. With fourteen years of cryo-EM and biochemistry experience, I am motivated to develop and combine new tools in purifying and reconstructing endogenous protein complexes at atomic resolution. As a postdoc, I was part of a collaborative effort that led to the discovery that defective products of translation are targeted by the Ribosome Quality Control (RQC) complex for proteasomal degradation by the Cdc48 AAA+ ATPase. My purification and cryo-EM analysis of native RQC particles via an accelerated “lysate-to-grid” approach led to our discovery of RQC-mediated peptide elongation reactions on dissociated, 60S ribosomes in a manner independent of mRNA (Shen *et al.*, Science 2015). Using an analogous approach, my independent group recently purified and imaged native, substrate-bound Cdc48 complexes, which led to a mechanistic model of protein unfolding by this essential molecular machine (Cooney *et al*. Science 2019). My success in using these approaches affirmed my conviction that top-down structural studies of cellular complexes assembled in vivo is a powerful approach to discovery biology.

My ongoing collaboration with Brenda Bass has been productive and led to the first molecular resolution structure of a metazoan Dicer (Sinha *et al.*, Science 2018). We now co-mentor a graduate student, Helen Donelick, who will spearhead the proposed studies to continue these efforts towards the goal of higher resolution structures of *Drosophila* Dicer and its accessory proteins. I am also excited about applying the “lysate-to-grid” methodology I developed to seek cryo-EM structures of the complexes involved in producing 26G endogenous siRNAs in *C. elegans*. The collaborative track record I have with the Bass Lab, and accessibility to our Titan Krios/K2 at the University of Utah, lends confidence that our work will lead to exciting new discoveries.

B. Positions and Honors

Positions and Employment

09/2015 - 07/2017 Director of Cryo-EM, University of Utah Electron Microscopy Core
09/2015 - 10/2017 Research Assistant Professor of Biochemistry, University of Utah School of Medicine
10/2017 – present Tenure Track Assistant Professor of Biochemistry, University of Utah School of Medicine

Other Experience

10/2018 – present NCCAT User Review Committee, National Center for CryoEM Access and Training, New York Structural Biology Center
01/2020 – present PNCC User Review Committee, Pacific Northwest Center for Cryo-EM, Pacific Northwest National Laboratory

Honors

2000-2003	Brigham Young University Multicultural Student Academic Award, Undergraduate
2007-2008	Brigham Young University Cancer Research Center Graduate Fellowship
2008-2009	Roland K. Robins Graduate Research Fellowship, Brigham Young University
2008	Loren C. & Maurine F. Bryner Outstanding Scholarship, Brigham Young University
2009	Albert D. & Jennie R. Swensen Outstanding Scholarship, Brigham Young University
2010-2011	Brigham Young University Graduate Research Fellowship
2017	Young Investigator Outstanding Recognition Award, FEI Thermo Fisher Scientific

C. Contribution to Science

1. Mechanism of eukaryotic co-translational quality control and discovery of non-templated elongation

The Ribosome Quality Control complex (RQC) is broadly conserved among eukaryotes and is required for clearing defective ribosomal products as a result of translating faulty mRNAs. As a postdoc in Adam Frost's lab, I designed studies that enabled the biochemical and structural characterization of the RQC, including the discovery that the Cdc48 AAA+ ATPase directly attaches to ribosomes to facilitate clearance or partially synthesized proteins (Brandman et al., 2012). I determined the cryo-EM structure of the Cdc48-free RQC particle, which revealed the mechanistic basis of how cells distinguish between ribosomes in a state of normal versus defective translation (Shen et al., 2015). My work revealed one class of isolated complexes with unexpected tRNA densities positioned within the 60S complex, in a manner reminiscent of peptide elongation, despite the absence of the 40S subunit. This structure led to our discovery that the RQC recruits charged alanine and threonine tRNAs to stalled 60S ribosomes, where they append C-terminal alanines and threonines (CAT tails) to partially synthesized proteins, which are then released and targeted for proteasomal degradation. More recently, we discovered that the Cdc48 adaptor Vms1 plays a direct role in cleaving CAT tails from stalled ribosomes (Zurita Rendón et al., 2018).

a) **Shen PS**, Park J, Qin Y, Parsawar K, Li X, Larson M, Cox J, Cheng Y, Lambowitz AM, Weissman JS, Brandman O, Frost A. Rqc2p and the 60S ribosome mediate mRNA-independent elongation of nascent chains. *Science*. 2015 Jan 2;347(6217):75-8. PMCID: PMC4451101
b) Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li GW, Zhou S, King D, **Shen PS**, Weibezahn J, Dunn JG, Rouskin S, Inada T, Frost A, Weissman JS. A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell*. 2012 Nov 21;151(5):1042-54. PMCID: PMC3534965
c) Zurita Rendón O, Fredrickson EK, Howard CJ, Van Vranken J, Fogarty S, Tolley ND, Kalia R, Osuna BA, **Shen PS**, Hill CP, Frost A, Rutter J. Vms1p is a release factor for the ribosome-associated quality control complex. *Nat. Commun.* 2018 Jun 6;9(1):2197. PMCID: PMC5989216

2. Structural basis of protein translocation by the AAA+ ATPases

The deeply conserved family of AAA+ ATPases span bacteria to humans and play fundamental roles in cell biology, including protein unfolding, protein degradation, intracellular trafficking, cell division, and so forth. AAA+ ATPases are characterized by their formation of hexameric rings with a central pore that functions as a translocation channel for substrate unfolding. I was part of a collaborative team that solved the first high-resolution structure of an asymmetric, substrate-bound AAA+ ATPase (Monroe et al., *eLife* 2017). In this study, we discovered that the Vps4 AAA+ ATPase uses a "hand-over-hand" mechanism of substrate translocation in which each of the six subunits translocate from the "bottom" of the assembly that surrounds the substrate to the top, thereby allowing the molecular machine to "crawl" along the substrate. In ensuing work, we improved the resolution of the complex, which enabled us to define the atomic-resolution details of Vps4-substrate interactions (Han et al., *eLife* 2017). Next, we extended our understanding of AAA+ mechanism by demonstrating the ability of Vps4 to translocate a circular peptide, thereby demonstrating that substrate translocation could occur on internal protein segments and branched chains, and that translocation is not restricted to terminal ends (Han et al., *eLife* 2019). Finally, my lab led the efforts to purify and image native complexes of the Cdc48 AAA+ ATPase. Cdc48 is essential, highly abundant, and of high clinical relevance because its mutations cause degenerative disease and its inhibition in cancer cells is emerging as a new route of cancer treatment. We demonstrated that Cdc48 uses an analogous hand-over-hand mechanism of substrate translocation as seen for Vps4 and other AAA+ ATPases (Cooney et al., *Science* 2019). This work establishes

a framework to understand how Cdc48 dysfunction causes degenerative disease and provide insights in the development of its inhibitors that have potential as therapeutic agents.

a) Monroe N, Han H, **Shen PS***, Sundquist WI*, Hill CP*. Structural Basis of Protein Translocation by the Vps4-Vta1 AAA ATPase. *Elife*. 2017 Apr 5;6. (*co-corresponding author) PMCID: PMC5413351
b) Han H, Monroe N, Sundquist WI*, **Shen PS***, Hill CP*. The AAA ATPase Vps4 binds ESCRT-III substrates through a repeating array of dipeptide-binding pockets. *Elife*. 2017 Nov 22;6 (*co-corresponding author) PMCID: PMC5716660
c) Han H, Fulcher JM, Dandey VP, Iwasa JH, Sundquist WI, Kay MS, **Shen PS***, Hill CP*. Structure of Vps4 with circular peptides and implications for translocation of two polypeptide chains by AAA+ ATPases. *Elife*. 2019 Jun 11;8 (*co-corresponding author) PMCID: PMC6602582
d) Cooney I, Han H, Stewart MG, Carson RH, Hansen DT, Iwasa JH, Price JC, Hill CP*, **Shen PS***. Structure of the Cdc48 segregase in the act of unfolding an authentic substrate. *Science*. 2019 Jun 27 (*co-corresponding author) PMID: 31249134

3. Mechanism of self vs. non-self recognition by the Dicer enzyme

Life depends on the ability to distinguish between self versus non-self. Invertebrates rely on the Dicer-2 enzyme to mount an anti-viral response in addition to its classical role in generating siRNAs to achieve RNA interference. In order to determine how Dicer distinguishes between self and non-self RNA, I collaborated with Brenda Bass to solve cryo-EM structures of Drosophila Dicer-2 alone and in complex with an RNA substrate. We discovered that Dicer uses two distinct domains on opposite ends of the protein to discriminate between self and non-self RNAs. More specifically, RNAs with blunt ends are recognized by a conserved, RIG-I-like helicase domain that is structurally similar to mammalian RNA receptors that induce an immune response. The helicase domain processively threads blunt, viral-like dsRNAs through the helicase domain and produces RNA products of various sizes. In contrast, the Platform-PAZ domain recognizes “self” dsRNAs harboring 3’ overhanging termini to produce canonical 22-nt siRNA products. Our work reveals a common mode of sensing viral RNAs among RIG-I-like helicases from flies to humans.

a) Sinha NK, Iwasa J, **Shen PS***, Bass BL*. Dicer Uses Distinct Modules for Recognizing dsRNA Termini. *Science*. 2018 Jan 19:359(6373):329-334. (*co-corresponding author) PMCID: PMC6154394

4. Molecular basis of Polycystic Kidney Disease pathogenesis

Autosomal dominant polycystic kidney disease (ADPKD) is the most commonly inherited, potentially life-threatening disorder in humans. ADPKD affects more than 12 million individuals worldwide, including ~600,000 Americans, and is the leading genetic cause of renal failure. The disease is characterized by the development of renal cysts that grow in number and size over time until the individual develops end stage renal disease at which point dialysis or transplantation becomes necessary. ADPKD is caused by mutations in either *PKD1* or *PKD2*, but the functions of their gene products are unknown. I worked alongside Erhu Cao to solve the atomic-resolution structure of PKD2, which revealed that the majority of pathogenic *PKD2* mutations are clustered in a single domain of the protein and that this polycystin domain is important for assembling the ion channel into its proper state. Our work provides a framework that informs structure-based drug discovery for the treatment of ADPKD.

a) **Shen PS***, Yang X*, DeCaen PG, Liu X, Bulkley D, Clapham DE, Cao E. The Structure of Polycystic Kidney Disease Channel PKD2 in Lipid Nanodiscs. *Cell*. 2016 Oct 20:167(3):763-73. (*co-first author) PMCID: PMC6055481

5. Structural evolution of viral capsids

As a graduate student, I used cryo-EM to perform comparative studies of evolutionarily related capsid structures. I solved and compared structures of mammalian versus avian polyomaviruses, which revealed key structural differences that produce different capsid structures, which likely contribute to their disparate etiologies. I also purified and solved the structure of a novel bacteriophage from the Great Salt Lake, which revealed deep conservation with an ancestral protein fold that is present in phages isolated from diverse aquatic environments.

a) **Shen PS**, Enderlein D, Nelson CD, Carter WS, Kawano M, Xing L, Swenson RD, Olson NH, Baker TS, Cheng RH, Atwood WJ, Johne R, Belnap DM. The structure of avian polyomavirus reveals variably sized capsids, non-conserved inter-capsomere interactions, and a possible location of the minor capsid protein VP4. *Virology*. 2011 Mar 1;411(1):142-52. PMCID: PMC3057058.

b) **Shen PS**, Domek MJ, Sanz-García E, Makaju A, Taylor RM, Hoggan R, Culumber MD, Oberg CJ, Breakwell DP, Prince JT, Belnap DM. Sequence and structural characterization of Great Salt Lake bacteriophage CW02, a member of the T7-like supergroup. *J. Virol*. 2012 Aug;86(15):7907-17. PMCID: PMC3421657

c) Thomas JA, Rolando MR, Carroll CA, **Shen PS**, Belnap DM, Weintraub ST, Serwer P, Hardies SC. Characterization of *Pseudomonas chlororaphis* myovirus 201φ2-1 via genomic sequencing, mass spectrometry, and electron microscopy. *Virology.* 2008 Mar; *376*(2):330–338. PMCID: PMC2577825

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/peter.shen.1/bibliography/public/

D. Research Support

Ongoing Research Support

R35 GM133772 (Shen, PI) 08/01/2019 – 05/31/2024
Visualizing the Mechanisms of Protein Quality Control
Major goals: The focus of this project is to perform structure-function analysis of the abundant and essential Cdc48/p97/VCP molecular machine. We aim to determine structures of Cdc48 in complex with substrates, regulatory binding partners, and stalled ribosomes. Our efforts will establish a framework to understand how Cdc48 dysfunction causes degenerative disease and provide insights in the development of its inhibitors that have potential as therapeutic agents.
Role: Principal Investigator

R25 EY029124 (Shen and Iwasa, MPIs) 05/01/2018 – 04/30/2021
Interactive, Self-Paced Training Modules for the Cryo-EM Novice
Major goals: Cryo-EM has emerged as an indispensable and increasingly popular method to visualize biological structures at high resolution. To aid the training effort of newcomers to the field, we will use a media-rich and user-friendly approach to create a self-paced, interactive online course that provides practical guidance for the cryo-EM novice. This course will be available at https://CryoEM101.org .
Role: Principal Investigator

R01 DK110575 (Cao, PI) 09/16/2016 – 07/31/2021
Structures and Mechanisms of Polycystic Kidney Disease Proteins
Major goals: To elucidate the structural principles and fundamental biophysical properties of polycystic kidney disease proteins, which are the sites of mutations that cause autosomal dominant polycystic kidney disease.
Role: Co-Investigator

Completed Research Support

R01 GM116560 (Hill and Formosa, MPIs) 05/01-2016 – 07/31/2019
Structure, Mechanism and Function of the Histone Chaperones Spt6 and FACT
Major goals: This project focuses on conserved histone chaperones that are essential for viability and also implicated in HIV latency. I am leading efforts to solve high-resolution cryo-EM structures of these chaperones in the context of their cognate binding partners.
Role: Co-Investigator

R01 GM121706 (Bass, PI) 09/01/2016 – 07/31/2019
Mechanistic insights into Dicer, a double-stranded RNA processing enzyme
Major goals: Dicer is an essential enzyme in all animals, where it cleaves double-stranded RNA (dsRNA) precursors to generate microRNAs (miRNAs) and small interfering RNAs (siRNAs). These small RNAs bind to messenger RNAs to regulate their expression. The proposed studies are focused on Dicer's helicase domain, which is most similar to a family of helicases involved in the innate immune response to viral infection. RIG-I, the founding member of this family, and Dicer, both recognize dsRNA termini. The goal of the proposed research is to understand how Dicer's helicase domain coordinates recognition of its dsRNA substrates, their termini and base-paired structures, to promote conformational changes that correlate with suboptimal (distributive) or optimal (processive) cleavage.
Role: Co-Investigator

P50 GM082545 (Sundquist, PI) 08/01/2017 – 07/31/2019

Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)
Structural Biology Core 2
Major goals: Our Structural Biology Core provides capabilities for visualizing structures of macromolecules, subcellular complexes, cells and tissues. My role is to provide support through determining structures by cryo-EM, including single-particle reconstructions and reconstructions of helical assemblies.
Role: Faculty Researcher

BIOGRAPHICAL SKETCH

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NAME: Brenda L. Bass

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

POSITION TITLE: Distinguished Professor

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
Colorado College, Colorado Springs, CO	B.A.	05/1977	Chemistry
University of Colorado, Boulder, CO	Ph.D.	08/1985	Chemistry
Fred Hutchinson Cancer Center, Seattle, WA	Postdoctoral	06/1989	Mol. & Dev. Biology

A. Personal Statement

Studies in my laboratory are focused on double-stranded RNA (dsRNA)--its biological functions and the proteins that bind it to mediate these functions. These studies are suited to my training, as well as expertise acquired from decades of research in my own laboratory on ADARs, Dicer, dsRNA, and dsRNA binding proteins (dsRBPs). As a postdoctoral fellow in the laboratory of the late Dr. Harold Weintraub, I discovered the dsRNA-specific RNA editing enzymes now known as ADARs, and this initiated my life-long interest in dsRNA. My laboratory was the first to show that the vast majority of ADAR editing sites within endogenous RNA are in noncoding rather than coding sequences, first using *C. elegans* (Morse & Bass, 1999), and then RNA isolated from mammalian brain (Morse et al., 2002). While these studies were controversial for many years, the ADAR field is now focused on editing in noncoding sequences, especially in regard to the mammalian innate immune response. My laboratory recently made an important contribution to this area by demonstrating that the primary function of ADARs in invertebrates is also to regulate the innate immune response (**a.** Reich et al., 2018).

My laboratory characterized the first animal lacking Dicer (Knight and Bass, 2001), and a subsequent microarray analysis of gene expression in this animal (*C. elegans*) showed that misregulated genes were enriched for those important to innate immunity (Welker et al., 2007). This led to our realization that Dicer's helicase domain is similar to a family of helicases that includes RIG-I, a mammalian protein involved in innate immunity, and initiated my interest in Dicer's helicase domain. My laboratory discovered that Dicer's helicase domain modulates catalysis in response to distinct "self" versus "nonself" dsRNA termini (Welker et al. 2011, Sinha et al. 2015), and in collaboration with Dr. Peter Shen, we used cryo-EM to provide mechanistic insight into these observations (**b.**, Sinha et al. 2018). Our in-depth characterization of Loqs-PD revealed that this dsRBP allows Dicer to become termini-independent, thus facilitating recognition of endogenous dsRNAs (**c.** Trettin et al., 2017). We have also accomplished our long-term goal of genome-wide mapping of expressed, long dsRNAs in *C. elegans*, mouse and human (Whipple, 2015; **d.** Blango, 2016). These "dsRNAomes" define "self" dsRNA and are allowing my lab and others to define the properties of long, endogenous dsRNA.

During my independent career I have trained 16 predoctoral graduate students and 19 postdoctoral fellows, the majority of whom have gone on to become successful independent scientists at universities, biotech companies or in other science-related endeavors. I made the decision to major in chemistry as an undergraduate in 1975, so I have been a woman in science for over 40 years. This experience has left me with no doubt about the importance of diversity to the pursuit of the best science. I revel in the diversity of the trainees in my current lab, and the 4 predoctoral students and two postdoctoral fellows highlight a mixture of gender and culture. In 2018 I attracted a Nigerian to my lab, and while not classified as a URM, there are few black scientists. In 2019 I was lucky to recruit a stellar Hispanic/Latino student to my lab. I take every opportunity to give these trainees the experiences they deserve, and truly enjoy seeing them excel.

In section C I review the significant contributions I have been involved in during my career, and below I cite significant publications in recent years.

a. Reich DP, Tyc KM, Bass BL. (2018). *C. elegans* ADARs antagonize silencing of cellular dsRNAs by the antiviral RNAi pathway. **Genes Dev**, 32, 271–82. PMCID: PMC5859968
b. Sinha NK, Iwasa J, Shen PS, Bass BL. (2018). Dicer uses distinct modules for recognizing dsRNA termini. **Science** (New York, NY). 359, 329–34. PMCID: PMC6154394
c. Trettin KD, Sinha NK, Eckert DM, Apple SE, Bass BL. (2017) Loquacious-PD facilitates Drosophila Dicer-2 cleavage through interactions with the helicase domain and dsRNA. **Proc Natl Acad Sci USA**, 114:E7939–48. PMCID: PMC5617286
d. Blango MG, Bass BL. (2016). Identification of the long, edited dsRNAome of LPS-stimulated immune cells. **Genome Research**, 26, 852–62. PMCID: PMC4889969

B. Positions and Honors

Positions and Employment

1989-1995	Assistant Professor, Dept. of Biochemistry, Univ. of Utah School of Medicine, Salt Lake City, UT
1995-1999	Associate Professor, Dept. of Biochemistry, Univ. of Utah School of Med., SLC, UT
1996-2003	Adjunct Associate Professor, Dept. of Human Genetics, Univ. of Utah School of Med., SLC, UT
1999-2007	Professor, Dept. of Biochemistry, Univ. of Utah School of Med., SLC, UT
1998-present	Member, Nuclear Control of Cell Growth & Differentiation Program, Huntsman Cancer Institute
2003-present	Adjunct Professor, Dept. of Human Genetics, Univ. of Utah School of Med., SLC, UT
2007-present	Distinguished Professor, Dept. of Biochemistry, Univ. of Utah School of Med., SLC, UT

Other Experience and Professional Memberships

1991	NIH Molecular Biology Study Section, Ad Hoc Member
1994-1999	*Current Biology*, Editorial Board Member
1995	Cold Spring Harbor RNA Processing Meeting, Co-organizer
1995-1996	RNA Society, Nominations Committee, Chair
1995-present	*RNA*, Editorial Board Member
1996	Gordon Research Conference on Nucleic Acids, Co-organizer
1998-1999	RNA Society, Council Member (elected office)
1998-2000	RNA Editing, Frontiers in Molecular Biology series, Oxford University Press, Volume Editor
1998-2002	*Nucleic Acids Research*, Editorial Board Member
1999-2001	University of Utah, Biological Chemistry Program, Director
2000	Damon Runyon Walter Winchell Postdoctoral Fellowships, Ad Hoc Reviewer
2001	Gordon Research Conference on RNA Editing, Co-vice Chair
2001-2004	NIH Cell Development and Function 2 Study Section, Member
2003	Gordon Research Conference on RNA Editing, Co-Chair
2004-2005	NIH Molecular Genetics C Study Section, Member
2004-2006	RNA Society, Board of Directors (elected office)
2004-2007	*Science*, Board of Reviewing Editors
2007	RNA Society, President (elected office)
2007-2010	AAAS, Council Delegate, Section on Biological Sciences, (elected office)
2009-2011	American Academy of Arts and Sciences, Class II, Section 1 panel member
2010	NIH Special Emphasis Panel/Scientific Review Group, ad hoc reviewer
2012	NIH Molecular Genetics A Study Section, ad hoc reviewer
2013-14	NIH New Innovator Award, Phase 1 Reviewer
2014	NIGMS Protein Structure Initiative Transition Planning Committee
2015	NIH New Innovator Award, Editorial Review Panel
2016	NIH New Investigator Maximizing Investigator Research Award (MIRA), reviewer
2017	NIH Molecular Genetics B Study Section, ad hoc reviewer
2017-present	Section Liaison, National Academy of Sciences, Section 21, Biochemistry
2019	National Academy of Sciences, Molecular Biology Award Committee
2019	24th Annual RNA Society Meeting, Krakow Poland, Co-Organizer
2019-2022	NIH Molecular Genetics B Study Section, Member

Honors

1983-1984	ARCS Recipient (Achievement Rewards for College Scientists)
1983-1984	University of Colorado Doctoral Fellowship
1985-1988	Damon Runyon-Walter Winchell Postdoctoral Fellowship
1990-1994	Pew Scholars Award
1991-1996	David and Lucile Packard Fellowship
2007-present	Distinguished Professor of Biochemistry, University of Utah School of Medicine
2007	American Academy of Arts & Sciences, elected member
2009-2019	H. A. and Edna Benning Presidential Endowed Chair
2010	Distinguished Scholarly and Creative Research Award, University of Utah
2011	NIH Director's Pioneer Award
2011	AAAS fellow (elected)
2015	National Academy of Sciences, elected member
2017	Honorary Doctor of Science, Colorado College, Colorado Springs
2019-present	Jon M. Huntsman Presidential Chair

C. Contributions to Science

1. Biological catalysis by RNA.

I was extremely fortunate to be introduced to the process of discovery as a graduate student in Dr. Tom Cech's laboratory during the discovery of ribozymes. This has given me a life-long love for the process of discovery, and the goal of passing on this tradition to my trainees. My main scientific contribution as a graduate student was to show that ribozymes use principles of catalysis that are similar to those of protein enzymes. In the final chapter of my thesis, I proposed a frame of reference for understanding ribozyme catalysis, based on experimental evidence, my knowledge of RNA structure, and known strategies of protein enzymes. This thesis chapter became part of an Annual Review of Biochemistry article that I co-authored with Dr. Cech.

1a. Bass BL, Cech TR. (1984). Specific interaction between the self-splicing RNA of *Tetrahymena* and its guanosine substrate: implications for biological catalysis by RNA. **Nature**, 308(5962), 820–6.

1b. Bass BL, Cech TR. (1986). Ribozyme inhibitors: deoxyguanosine and dideoxyguanosine are competitive inhibitors of self-splicing of the *Tetrahymena* ribosomal ribonucleic acid precursor. **Biochemistry**, 25(16), 4473–7.

1c. Cech TR, Bass BL. (1986). Biological catalysis by RNA. **Annual Review of Biochemistry**, 55, 599–629.

2. Discovery and biochemical characterization of ADAR RNA editing enzymes.

As a postdoctoral fellow in the laboratory of the late Dr. Harold Weintraub, I discovered the RNA editing enzymes now known as Adenosine deaminases that act on RNA, or ADARs. Work on ADARs in my own laboratory led to many observations that proved significant to the field. Among the contributions that advanced knowledge of ADAR biochemistry are the first demonstration that catalysis involved a hydrolytic deamination of adenosine (collaboration with J. McCloskey), and the first structure of an ADAR, namely, the 1.7Å x-ray crystal structure of the catalytic domain of human ADAR2 (collaboration with C. Hill); serendipitously, this study identified an essential ADAR cofactor, inositol hexakisphosphate. We were the first to characterize the 5' nearest neighbor preferences of ADARs, which have been key for numerous studies of ADAR editing sites *in vivo*. A graduate student spearheaded a screen that identified mutations that altered ADAR preferences, and these studies unexpectedly showed that preferences relate to the base-flipping mechanism of ADARs (Kuttan & Bass, 2012).

2a. Bass BL, Weintraub H. (1988). An unwinding activity that covalently modifies its double-stranded RNA substrate. **Cell**, 55(6), 1089–98.

2b. Polson AG, Crain PF, Pomerantz SC, McCloskey JA, Bass BL. (1991). The mechanism of adenosine to inosine conversion by the double-stranded RNA unwinding/modifying activity: a high-performance liquid chromatography-mass spectrometry analysis. **Biochemistry**, 30(49), 11507–14.

2c. Macbeth MR, Schubert HL, Vandemark AP, Lingam AT, Hill CP, Bass BL. (2005). Inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing. **Science**, 309(5740), 1534–9. PMCID: PMC1850959

2d. Kuttan A, Bass BL. (2012). Mechanistic insights into editing-site specificity of ADARs. **Proc Natl Acad Sci USA**, 109(48), E3295–304. PMCID: PMC3511710

3. The biological role of ADARs.

My laboratory has also contributed to the understanding of the biological function of ADARs. We were the first to demonstrate that ADARs target hepatitis delta virus to change a stop codon to a tryptophan, and that this is crucial to the life cycle of the virus (collaboration with J. Casey). We were also the first to show that the vast majority of inosines within endogenous RNA are in noncoding rather than coding sequences using RNA isolated from *C. elegans* (Morse & Bass, 1999) and mammalian brain (Morse et al., 2002). Subsequently we showed that *C. elegans* with deletions in their ADAR genes have chemotaxis defects (collaboration with M. Krause). While initial reports suggested that all transcripts containing non-selective editing in mRNA noncoding sequences caused nuclear-retention, my laboratory showed that, at least in some cases, such mRNAs are loaded onto polysomes and translated (Hundley et al., 2008).

3a. Polson AG, Bass BL, Casey JL. (1996). RNA editing of hepatitis delta virus antigenome by dsRNA-adenosine deaminase. **Nature**, 380(6573), 454–6.

3b. Morse DP, Bass BL. (1999). Long RNA hairpins that contain inosine are present in *Caenorhabditis elegans* poly(A)+ RNA. **Proc Natl Acad Sci USA**, 96, 6048–53. PMCID: PMC26833

3c. Tonkin LA, Saccomanno L, Morse DP, Brodigan T, Krause M, Bass BL. (2002). RNA editing by ADARs is important for normal behavior in *Caenorhabditis elegans*. **EMBO J**, 21(22), 6025–35. PMCID: PMC137199

3d. Hundley HA, Krauchuk AA, Bass BL. (2008). *C. elegans* and *H. sapiens* mRNAs with edited 3' UTRs are present on polysomes. **RNA**, 14(10), 2050–60. PMCID: PMC2553745

4. dsRNA binding proteins and RNA interference.

I have been fascinated with the unique properties of dsRBPs for many years. When I was a new Assistant Professor, a rotation student in my lab conducted a screen for dsRBPs (Bass et al. 1994). Although we were not the first to publish, this study revealed what is now known as the dsRNA-binding motif (dsRBM). In this study, using the dsRBM in database searches, we also provided the first documentation of the enzyme now known as Dicer (*C. elegans*, K12H4.8). Years later this allowed me to write a Cell minireview that predicted that the genes encoding what is now known as Dicer would be key to this pathway (Bass, 2000). The latter accelerated progress in the field, and allowed my laboratory and others to prove that Dicer is the key enzyme in RNA interference (RNAi; Knight and Bass, 2001). In subsequent years we discovered that certain dsRBPs involved in dsRNA-mediated gene silencing use cooperativity to acquire higher affinity for longer dsRNA (Parker et al., 2006), and that the existence of this property correlates with the function of the dsRBP (Parker et al. 2008).

4a. Bass BL, Hurst SR, Singer JD. (1994). Binding properties of newly identified Xenopus proteins containing dsRNA-binding motifs. **Curr Biol**, 4(4), 301–14.

4b. Bass BL. (2000). Double-stranded RNA as a template for gene silencing. **Cell**, 101(3), 235–8.

4c. Knight SW, Bass BL. (2001). A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. **Science**, 293(5538), 2269–71. PMCID: PMC1855227

4d. Parker GS, Maity TS, Bass BL. (2008). dsRNA binding properties of RDE-4 and TRBP reflect their distinct roles in RNAi. **J Mol Biol**, 384(4), 967–79. PMCID: PMC2605707

5. Intersection of RNA editing and RNA interference.

Many studies of my laboratory reiterate that dsRBPs are not sequence specific. Early on in these studies we considered the possibility that the lack of sequence specificity allowed dsRNA-mediated pathways to intersect and affect each other. Indeed, my laboratory was the first to show that deletions of ADAR genes cause aberrant dsRNA-mediated gene silencing (Knight & Bass, 2002; Tonkin & Bass, 2003). We also showed that an abundant, long, non-coding dsRNA could sequester Dicer from its own dsRNA substrates (Hellwig & Bass, 2008), and that *C. elegans* strains lacking ADARs have altered levels of small RNAs, such as miRNAs (Warf et al., 2012).

5a. Knight SW, Bass BL. (2002). The role of RNA editing by ADARs in RNAi. **Mol Cell**, 10(4), 809–17.

5b. Tonkin LA, Bass BL. (2003). Mutations in RNAi rescue aberrant chemotaxis of ADAR mutants. **Science**, 302(5651), 1725. PMCID: PMC1850956

5c. Hellwig S, Bass BL. (2008). A starvation-induced noncoding RNA modulates expression of Dicer-regulated genes. **Proc Natl Acad Sci USA**, 105(35), 12897–902. PMCID: PMC2519042

5d. Warf MB, Shepherd BA, Johnson WE, Bass BL. (2012). Effects of ADARs on small RNA processing pathways in C. elegans. **Genome Research**, 22(8), 1488–98. PMCID: PMC3409262

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/brenda.bass.1/bibliography/public/

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

Ongoing Research Support

R01 GM044073-25 (Bass, PI) 04/01/1990 – 05/31/2021
NIH NIGMS
The Biology and Biochemistry of ADAR RNA Editing Enzymes
ADAR RNA editing enzymes have established functions in "editing" mRNA codons to allow multiple protein isoforms. However, recoding sites are rare, and there are far more editing sites in noncoding regions of mRNAs. Little is known about the functions of editing in noncoding sequences. Proposed studies are designed to fill this gap in knowledge, possibly revealing regulatory pathways important for innate immunity and gene silencing.
Role: Principle Investigator

R01CA260414-01 (Bass, co-PI) 09/01/2020 – 08/31/2025
NIH, NCI
Unlocking evolutionarily latent immune functions for treating disease
Harnessing immune pathways to attack a diseased state, or immunotherapy, is a proven and powerful way to treat disease such as cancer. Prior immunotherapies focus on manipulation of the adaptive immune response, but the focus of these studies is on innate immune pathways. There are many natural checkpoints in place to prevent an aberrant innate immune response. Proposed studies are designed to identify and modulate innate immune checkpoints to reveal previously unsuspected ways to exploit innate immune pathways to treat disease. This is a multi-PI project that includes Dr. Nels Elde (University of Utah), Dr. Jane Jackman (Ohio State University) and Dr. Dan Stetson (University of WA). Dr. Peter Shen is a co-Investigator on this project.

R35GM141262-01 (Bass, PI) 04/01/2021-03/31/2026
NIH, NIGMS
Elucidating Roles and Mechanisms of Double-Stranded RNA-Mediated Pathways
This MIRA application is designed to encompass and replace two NIGMS R01s, one ongoing (GM044073-25) and one completed (GM121706-04); while funding decisions are forthcoming, the application was positively reviewed (impact score, 22). The proposed research will use the model organism *C. elegans*, biochemistry, and structural biology, to elucidate pathways and mechanisms involved in distinguishing “self” from “nonself” dsRNA, including those involving the dsRNA binding proteins ADAR and Dicer.
Role: Principal Investigator

Completed

R01 GM121706-04 (Bass, PI) 01/01/2017 – 12/31/2020
NIH NIGMS
Mechanistic Insights into Dicer, a Double-Stranded RNA Processing Enzyme
This grant focused on Dicer, an enzyme essential for life. Studies used biochemical and structural methods to reveal how Dicer recognizes its double-stranded RNA (dsRNA) substrates and distinguishes foreign dsRNA from naturally-occurring dsRNA.
Role: Principal Investigator

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: Helen Donelick

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

POSITION TITLE: Graduate Research Assistant (PhD Candidate)

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Pacific University, Forest Grove, OR	B.S.	08/2013	05/2017	Biology
University of Utah, Salt Lake City, UT	PhD	08/2017		Biochemistry

A. Personal Statement

My long-term research interest involves the interface between RNA biology and the use of cryo-EM to study biochemical reactions. I was drawn to this field as RNA biology is a challenge, we know so little about RNA biology, yet it plays a major role in all organisms. Additionally cryo-EM allows us to visualize and solve the structure of proteins, and this field is still growing at a rapid rate, permitting me to be on the forefront of a developing technology. I am always willing to face a challenging project and my enthusiasm for basic scientific research, makes me uniquely qualified to study my project. My academic training at Pacific University, the University of Utah, and my research experience to date, have provided an excellent background in molecular biology. As an undergraduate at Pacific University, I conducted research with Dr. Joanne Odden on the infection rates of *Wolbachia* in *Drosophila melanogaster*, and the effect of *Wolbachia* infections on cytoplasmic incompatibility. This resulted in a publication, as well as the opportunity to present my research at conferences and symposiums throughout my undergraduate career. I also was selected as a NSF-sponsored Research Experience for Undergraduates (REU) scholar at the University of Utah in Dr. Cynthia Burrows laboratory. In this lab, I focused on the bisulfite reaction on cytidine modifications. I had the exciting opportunity to give an oral presentation at the American Chemical Society (ACS) conference in San Francisco in April 2016, in the session, REU: Chemistry in Action; only ten students were selected nationwide to give talks in this session. I have now moved into the fields of RNA-protein biochemistry and structural biology, by studying the double-stranded RNA binding protein, Dicer-2, under the supervision of Dr. Brenda Bass and Dr. Peter Shen. Dr. Bass is an internationally recognized leader in the field of RNA biology, and Dr. Shen is at the cutting edge of the cryo-EM revolution, being among the elite group of young scientists who were already engaged in cryo-EM studies when it came of age. For my initial project, I am currently exploring methods in which to study Dicer-2 structurally, along with key substrates and co-factors. Overall, through my choice of sponsor, co-sponsor, research project, and training from this fellowship, I can develop a strong foundation in my chosen fields. This will aid my long-term goal of working as a facilities manager of a cryo-EM facility.

B. Positions and Honors

Positions and employment:

2014 - 2017	Undergraduate research student, Pacific University
2018 - present	Graduate student (PhD candidate), University of Utah

Other Experience and Professional Memberships

2017 - present	Member, Tri-Beta honors society
2018 - present	Member, RNA society

Honors

2016	NSF sponsored REU scholar
2016	Portland American Chemical Society symposium, first place poster
2017	Biology Achievement award
2018	Program for Interdisciplinary Training in Chemical Biology, T32 grant (PITCH) trainee
2021	Student scholarship from Keystone Symposium: Free registration

C. Contributions to Science

1. **Undergraduate Research:** I was part of a project in the laboratory of Dr. Joanne Odden at Pacific University. Dr. Odden's laboratory studies the microbiology of *Wolbachia* infections in *Drosophila melanogaster*, and other invertebrates in the Pacific Northwest. During my time in her lab, I was studying at the infection rates of *Wolbachia* in *Drosophila melanogaster,* collected in areas near the university, and testing whether *Wolbachia* infections resulted in cytoplasmic incompatibility in *Drosophila* embryos. My contributions to this work were included in a publication recently accepted in *Western North American Naturalist*. The work was particularly exciting because it provides some of the first data on *Wolbachi*a infection rates in Western North America.
    a. Odden J.P., Eng, W., Lee, K., **Donelick H**., Hiefield M., Steach J., Chan, L. (2019) Novel host-bacterial symbioses revealed: Characterization of *Wolbachia* in arthropods of western North America. *Western North American Naturalist*.

   Additionally, I had the opportunity to complete research at the University of Utah in Dr. Cynthia Burrows laboratory in the Department of Chemistry, as a NSF-sponsored REU scholar. Here I studied the bisulfite reaction with two different modifications on cytidine. After completing my internship I was selected to present an oral presentation at the national ACS conference in April 2017.
    a. **Donelick, H**., Rodgers A., Burrows, C. Understanding the Products of the Bisulfite Reaction with Two Types of Cytidine. ACS national conference, REU Chemistry in Action; April 2017; San Francisco, CA.

2. **Graduate Research**: My ongoing predoctoral research is focused on structural and functional understanding of the RNA interference pathway in *Drosophila melanogaster*. My main project focuses on Dicer-2, a key protein in the RNA interference pathway, and this protein's key co-factors and substrates. I use biochemistry and cryo-EM to relate the biochemical function to the structure of the protein and protein-RNA complexes. The results from my research are relevant to human health as information on the RNAi pathway in a model organism can give insight into antiviral defense. I had the chance to present several posters on my current progress at the RNA Society meeting, an international conference in 2019 in Krakow, Poland, the virtual Cold Spring Harbor conference in May 2020, and the virtual Keystone symposium: Frontiers in Cryo Electron Microscopy in February 2021. I was a author on a recent review published by Cold Spring Harbor regarding the Dicer helicase domain. Finally, I am co-first-author paper on a paper published *RNA.*

a. **Donelick HM**., Bass BL., and Shen PS. Structural Determination of Dicer-2 Complexes. RNA Society Conference; June 2019; Krakow, Poland.

b. Hansen SR., Aderounmu AM., **Donelick HM**., Bass BL. Dicer's Helicase Domain: A Meeting Place for Regulatory Proteins. *Cold Spring Harbor Symposia on Quantitative Biology* 84: doi:10.1101/sqb.2019.84.039750.

c. **Donelick HM**, Talide L, Bellet M, Aruscavage J, Marques JT, Imler JL, Shen PS, Meignin C, Bass BL. dmDcr-2 helicase domain is essential for viral and endogenous dsRNA processing. Cold Spring Harbor Conference on Regulatory and Non-coding RNAs; May 2020; virtual conference.

d. Donelick HM and Talide L, Bellet M, Aruscavage J, Lauret E, Aguiar E, Marques JT, Meignin C, Bass BL. In vitro studies provide insight into effects of Dicer-2 helicase mutations in *Drosophila melanogaster*. *RNA*. 2020.

e. **Donelick HM.,** Bass BL., and Shen PS. Structural determination of the Dicer-2•R2D2 complex. Keystone symposia: Frontiers in cryo-EM; February 2021; virtual conference.