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 central theme of my research program is to define how molecular machines work. As a postdoc with Adam Frost, 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. During that time, advances in cryo-EM opened the floodgates for high-resolution structure determination, which I used to discover a process in which the RQC complex performs peptide synthesis reactions on dissociated, 60S ribosomes in a manner independent of mRNA (Shen *et al.*, Science 2015). This discovery was possible because of our approach to characterize native complexes and computationally sort through inherent heterogeneity, and this work affirmed my conviction that top-down studies of endogenous cellular complexes can provide valuable structural and mechanistic insights.

Following my postdoc, I accepted a research faculty position in 2015 that enabled me to establish new collaborations while developing my independent research program. In this capacity, I led collaborative efforts to resolve cryo-EM structures of other challenging macromolecular complexes that contributed to the award of three R01 grants, an award from the Beckman Foundation to establish a world-class cryo-EM facility, and high-impact publications, including the 3.0 Å resolution structure of the Polycystic Kidney Disease channel PKD2 (Shen *et al*. Cell 2016), structures of the Vps4 AAA ATPase (Monroe *et al*. eLife 2017, Han *et al.* eLife 2017), and structures of the Dicer-2 RNA processing enzyme (Sinha *et al.* Science 2018). My commitment to providing training is also reflected our recent R25 award (with Janet Iwasa) to develop a web-based “CryoEM 101” course for newcomers to the field. Altogether, these efforts demonstrated my leadership ability to carry out projects and motivated me to start an independent lab to pursue my interests in studying the structural landscapes of molecular machines.

In 2017, I accepted a tenure track faculty appointment in the same department and have continued my collaboration with Brenda Bass to study the mechanisms of how the Dicer enzyme processes RNA substrates. As described in this proposal, we now seek to extend our published work by solving atomic-resolution structures of Dicer-RNA complexes. Access to the Chameleon and Titan Krios/K2 instrumentation at NYSBC NCCAT will enable us to prepare optimal samples and collect the necessary data to achieve these goals.

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	Tenure Track Assistant Professor of Biochemistry, University of Utah School of Medicine

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). To accomplish this, I developed and optimized an accelerated 'lysate-to-grid' strategy that allowed me to isolate native RQC particles from budding yeast and immobilize them on cryo-EM grids within the span of a few hours. Datasets from my native RQC particles revealed extensive compositional heterogeneity, which I was able to deconvolve using 3D classification approaches. 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 prompted us to investigate tRNAs binding to the RQC and 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. These studies provided the first example of non-templated ribosomal protein synthesis, and affirmed my conviction that top-down studies of endogenous cellular complexes can provide deep structural and mechanistic insights. In this case, we discovered unexpected new biology because we were able to directly visualize complex ensembles of native assemblies without prior knowledge of all of their components.

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 Vps4 AAA ATPase

Many membrane remodeling events, such as intracellular trafficking, retroviral budding, and cell division, are dependent on the formation of ESCRT-III polymers. The Vps4 AAA ATPase uses energy from ATP hydrolysis to break apart ESCRT-III polymers and trigger membrane fission. In order to understand how Vps4 works, I solved cryo-EM structures of the enzyme bound to an ESCRT-III peptide substrate. To accomplish this, I employed signal subtraction with focused refinement to clarify conformational heterogeneity and help to define the catalytic mechanism of an asymmetric, substrate-bound Vps4 AAA ATPase (a collaboration with Chris Hill and Wes Sundquist). These focused refinements improved the resolution and allowed us to model the multiple conformational states adopted by a mobile protomer within the asymmetric, helical Vps4 hexamer. This

analysis supported a “hand-over-hand” mechanism in which Vps4 subunits translocate from the “bottom” of the Vps4 helix that surrounds the substrate to the top, thereby allowing the enzyme to “crawl” along the polypeptide substrate (which is topologically equivalent to pulling the substrate through the central pore of the hexamer). This mechanism may be generally applicable to other AAA proteins, including p97/Cdc48.

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

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) PMID: 29269422

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/browse/collection/41842444/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

1 R25 EY029124-01 (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

1 R01 GM116560-01A1 (Hill and Formosa, MPIs) 05/01-2016 – 04/30/2020
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

1 R01 DK110575-01A1 (Cao, PI) 09/01/2016 – 08/31/2021
Structures and Mechanisms of Polycystic Disease Proteins
Major goals: The goal of this project is 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 (ADPKD). Treatment options for this prevalent genetic disorder are currently limited, in large part because the molecular mechanisms of the relevant proteins are only poorly understood. Successful outcome of this project will provide structural and biochemical insights that will inform the development of novel therapeutic strategies.
Role: Co-Investigator

1 R01 GM121706-1 (Bass, PI) 09/01/2016 – 08/31/2021
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

5 P50 GM082545-11 (Sundquist, PI) 08/01/2017 – 07/31/2022
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

Completed Research Support

None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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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/Biochemistry
Fred Hutchinson Cancer Center, Seattle, WA	Postdoctoral	06/1989	Mol. & Dev. Biology

A. Personal Statement

The goal of the proposed research is to gain a mechanistic understanding of how Dicer recognizes its double-stranded RNA (dsRNA) substrates. This goal is suited to my training, as well as expertise acquired from over 25 years of working with dsRNA and dsRNA binding proteins (dsRBPs). My training in RNA biochemistry began in the laboratory of Dr. Tom Cech where I performed the first kinetic analyses of ribozymes. 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 long-term interest in dsRNA. Work on ADARs and other dsRBPs in my own laboratory led to many observations that proved significant to the field (see Part C), and gave me expertise relevant to the proposed work on Dicer.

My entry into the dsRNA-mediated gene silencing field capitalized on my expertise and knowledge about dsRNA, and 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 our work led to important paradigms about dsRBPs involved in RNAi (see Part C). My laboratory discovered that Dicer's helicase domain modulates catalysis in response to different dsRNA termini (Welker et al. 2011; Sinha et al. 2015). Most recently, in collaboration with Dr. Peter Shen, we used cryo-electron microscopy to solve structures of *Drosophila* Dicer-2 alone and in complex with blunt dsRNA. Whereas the Platform-PAZ domains have been considered the only Dicer domains that bind dsRNA termini, unexpectedly, we found that the helicase domain is required for binding blunt, but not 3' overhanging, termini. We further showed that blunt dsRNA is locally unwound and threaded through the helicase domain in an ATP-dependent manner. These studies revealed a previously unrecognized mechanism for optimizing antiviral defense and set the stage for the discovery of helicase-dependent functions in other Dicers.

1. 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
2. Welker NC, Maity TS, Ye X, Aruscavage PJ, Krauchuk AA, Liu Q, Bass BL. (2011). Dicer's helicase domain discriminates dsRNA termini to promote an altered reaction mode. **Mol Cell**, 41(5), 589–99. PMCID: PMC3061311
3. Sinha NK, Trettin KD, Aruscavage PJ, Bass BL. (2015). Drosophila Dicer-2 cleavage is mediated by helicase- and dsRNA termini-dependent states that are modulated by Loquacious-PD. **Mol Cell**, 58(3), 406–17. PMCID: PMC4433149
4. Sinha NK, Iwasa J., Shen PS, Bass BL (2018). Dicer uses distinct modules for recognizing dsRNA termini. **Science**, 359(6373), 329–334. PMCID: PMC6154394

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

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-present American Academy of Arts & Sciences (elected member)
2009-present 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-present National Academy of Sciences, elected member
2017 Honorary Doctor of Science, Colorado College, Colorado Springs

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). My laboratory was the first to show that the vast majority of inosines 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 this observation was controversial at first report, many in the RNA editing field now study editing in noncoding sequences and its relationship to innate immunity. 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, Aruscavage PJ, Bass BL. (2002). RNA hairpins in noncoding regions of human brain and *Caenorhabditis elegans* mRNA are edited by adenosine deaminases that act on RNA. **Proc Natl Acad Sci, USA**, 99(12), 7906–11. PMCID: PMC122993
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 the dsRNAome.

I have been fascinated with the unique properties of dsRBPs for many years. As 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 *C. elegans* Dicer (K12H4.8). 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). Spurred by a Pioneer Award, in recent years we accomplished our long-term goal of the genome-wide mapping of expressed, long dsRNAs in *C. elegans*, mouse and human.

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. 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
4c. Whipple JM, Youssef OA, Aruscavage PJ, Nix DA, Hong C, Johnson WE, Bass BL. (2015). Genome-wide profiling of the *C. elegans* dsRNAome. **RNA,** 21, 786–800. PMCID: PMC4408787
4d. Blango MG, Bass BL. (2016). Identification of the long, edited dsRNAome of LPS-stimulated immune cells. **Genome Research**, 26, 852–62. PMCID: PMC4889969.

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). Our most recent progress in this area reveals that in *C. elegans*, editing of cellular dsRNA by ADARs marks them as "self", thus precluding their recognition by Dicer and an antiviral response (Reich et al., 2018).

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. 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
5d. 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

Complete List of Published Work in MyBibliography (for accuracy, please paste URL into browser):
https://www.ncbi.nlm.nih.gov/sites/myncbi/1Rwz2MvKcbnkJ/bibliography/44346563/public/?sort=date&direction=descending

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

Ongoing Research Support

R01 GM121706-02 (Bass, PI) 01/01/2017 – 12/31/2020
NIH NIGMS
Mechanistic Insights into Dicer, a Double-Stranded RNA Processing Enzyme
This grant focuses on Dicer, an enzyme essential for life. Studies will use 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

R01GM044073-23 (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

Completed Research Support (last 3 years)

8DP1AG044162-05 (Bass, PI) 09/30/2011-07/31/2016
NIA (NIH Office of the Director)
Cellular Double-stranded RNA as a Signal of Stress, Immunity, and Aging
This Pioneer Award was aimed at revealing a previously unrecognized network of cellular double-stranded RNA signaling molecules responsible for triggering pathways implicated in stress, immunity and longevity. A key outcome of these studies was the assembly of maps of the dsRNAs expressed in *C. elegans*, mouse and human. These dsRNAomes serve as a resource for my laboratory and others.
Role: Principle Investigator