PI: **Higuchi-Sanabria, Ryo**	Title: More than just a load control: cytoskeletal form and function during aging	
Received: 07/01/2019	FOA: PA19-130 Clinical Trial:Not Allowed	Council: 01/2020
Competition ID: FORMS-E	FOA Title: NIH Pathway to Independence Award (Parent K99/R00 - Independent Clinical Trial Not Allowed)	
1 K99 AG065200-01A1	Dual:	Accession Number: 4324404
IPF: 577502	Organization: UNIVERSITY OF CALIFORNIA BERKELEY	
Former Number:	Department: Molecular and Cell Biology	
IRG/SRG: NIA-B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 112,750 Year 2: 112,750 Year 3: 249,000 Year 4: 249,000 Year 5: 249,000	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Ryo Higuchi-Sanabria	The Regents of the University of California	PD/PI
Andrew Dillin	The Regents of the University of California	Other (Specify)-Sponsor
Barbara Meyer	University of California, Berkeley	Other (Specify)-Co-Sponsor

Reference Letters

[redacted]

Additions for Review

Updated Pages post submission material

OMB Number 4040-0001
Expiration Date 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ● Application ○ Changed/Corrected Application

4.a. Federal Identifier [redacted]

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: The Regents of the University of California
Department: Sponsored Projects Office
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:

Person to be contacted on matters involving this application
Prefix: First Name*: Margaret Middle Name: Last Name*: Nguyen Suffix:
Position/Title: Contract and Grant Officer
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

○ New ● Resubmission
○ Renewal ○ Continuation ○ Revision

If Revision, mark appropriate box(es).
○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)* :

Is this application being submitted to other agencies?* ○Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
More than just a load control: cytoskeletal form and function during aging

12. PROPOSED PROJECT
Start Date* 04/01/2020
Ending Date* 03/31/2025

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-013

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Ryo Middle Name: Last Name*: Higuchi-Sanabria Suffix:
Position/Title: Postdoctoral Researcher
Organization Name*: The Regents of the University of California
Department: Molecular and Cell Biology
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$990,540.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$990,540.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Margaret Middle Name: Last Name*: Nguyen Suffix:
Position/Title*: Contract and Grant Officer
Organization Name*: The Regents of the University of California
Department: Sponsored Projects Office
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative* Margaret Nguyen

Date Signed* 07/01/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_Higuchi-Sanabria_R1.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The Regents of the University of California
Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: CA-013

Additional Location(s) File Name:

Funding Opportunity Number: PA-19-130. Received Date: 2019-07-01T16:26:57.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Summary_R1.pdf
8. Project Narrative*	Project_Narrative.pdf
9. Bibliography & References Cited	References_Cited_R1.pdf
10.Facilities & Other Resources	Facilities_and_Resources_R1.pdf
11.Equipment	Equipment_R1.pdf

Funding Opportunity Number: PA-19-130. Received Date: 2019-07-01T16:26:57.000-04:00

Project Summary. Although the cytoskeleton has historically been understood as the structural framework of the cell, the proper function of actin is also required for a diverse array of cellular pathways. The collapse of these cellular processes manifests during aging and exposure to a myriad of stresses, which is in part due to the breakdown of the cytoskeleton under these conditions. Interestingly, the breakdown of the cytoskeleton throughout age has been adopted as common knowledge in the field of aging biology, despite the lack of clear and direct evidence. A major contributor to the lack of these essential studies is the lack of tools available for *in vivo*, live-cell imaging of the actin cytoskeleton in multi-cellular organisms. Early in my postdoctoral career, I developed a system for robust, tissue-specific, live-cell imaging of the cytoskeleton in the muscle, intestine, and hypodermis of *C. elegans*, utilizing LifeAct fused to a fluorescent molecule. LifeAct-mRuby reliably binds to F-actin, allowing visualization of functional, filamentous actin in the cells it is expressed. Using this system, I performed an exhaustive characterization of the decline of actin cytoskeletal integrity during aging.

This work laid the foundation of my currently ongoing work in identification of novel regulators of the actin cytoskeleton. Having set up a system to interrogate cytoskeletal quality, I can now interrogate novel genes in their potential role for actin regulation. Using this and other platforms, I performed a multi-pronged screening approach to identify novel genetic regulators of actin. These studies combined *in vivo* live cell imaging of actin filaments, synthetic lethality screening with known regulators of the actin cytoskeleton, and both transcriptome analysis and whole genome CRISPR-Cas9 screening of organisms experiencing actin stress. Cross-referencing these rich datasets has revealed two critical nodes of genes: 1) modifiers of chromatin state and their downstream transcriptional regulators and 2) genes involved in lipid storage and global lipid homeostasis.

In Aim 1.1, I hypothesize that a general chromatin state exists to promote a healthy transcriptome for proper cytoskeletal form and function, and that this breaks down as a function of age. Moreover, a healthy metabolic state can work either upstream of – or independent of – chromatin remodeling to also promote cytoskeletal health. In Aim 1.2, I propose to study whether any of the identified processes can function in a tissue-specific manner and a cell non-autonomous manner, by answering two questions: 1) is overexpression of chromatin remodeling or lipid homeostasis factors in a single tissue sufficient to preserve organismal lifespan? and 2) does overexpression of these genes in neurons drive protection of the actin cytoskeleton in peripheral tissue? Aim 2 uses 2 biochemical approaches to assess cytoskeletal function. First, proximity labeling will be used to characterize novel protein interactors of actin important for proper form and function. Second, we are building a tool for a biochemical approach for quantifying actin function with single cell resolution. This study will open exciting avenues of research in understanding the role of cytoskeletal function on physiological aging.

Many cellular functions, such as autophagy, organelle dynamics, and endocytosis/exocytosis, as well as their dedicated quality control machineries, such as the ubiquitin-proteasome system and the heat-shock response, decline in efficiency and function during the aging process. The actin cytoskeleton is no exception, and exhibits marked decline in structural integrity and function at old age. I propose a multipronged approach to understand how the regulatory network involved in cytoskeletal maintenance deteriorates during aging, and how this contributes to the physiological consequences of aging.

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Facilities and Resources:

Laboratory:
The Dillin lab is located in the Li Ka Shing Center on the University of California, Berkeley (UCB) Campus. The main laboratory is approximately 2000 sq. ft. and is devoted to bench and desk space for laboratory personnel. The space is adequate for 22 full-time researchers, and is fully equipped for cell biology, biochemistry, and molecular biology. Next door to the lab space is a microscope room (125 sq. ft.), a tissue culture room (200 sq. ft.), a chemical room (100 sq. ft.), an equipment room (100 sq. ft.), a shared cold room, two additional shared equipment rooms, and a shared warm room. There is also a shared media center in a separate building staffed by full-time personnel, and which houses automated plate pourers that together are capable of readily providing all of the plates and media required for the work in this proposal.
The Meyer lab is located in Koshland Hall, neighboring the Li Ka Shing Center and has 3320 sq. ft. of renovated lab space, including benches and desks for 21 scientists, 3 instrument rooms, 3 microscope rooms, and 1 cold room.

Clinical:
Not Applicable.

Animal:
N/A

Computer:
Every member of the Dillin lab has been equipped with his/her own Macintosh computer or PC and has designated non-bench desk space for use. Additional computers are available in the principal investigator's office, in a bioinformatics work space, in the microscope rooms, and in the equipment room. All the computers are fully equipped for word and image processing and have full access to the Internet via Ethernet connections as well as to an UCB-wide wireless network. All researchers are given VPN access in order to work remotely when required. The computers are on a network and share files through a server provided by UCB. UC Berkeley also provides on-demand access to journals and databases.
The Meyer lab has a 144 sq ft. computer room that is equipped with 4 Linux servers, 2 Linux workstations, 3 Apple MacIntosh computers and a Windows computer. Linux workstations have 16 to 32 CPU cores each and 64 to 128 GB of RAM installed, enabling them to handle the large data sets that will be obtained by the proposed work. The file servers host a total of 173 TB of storage space and are capable of reading data at 1.5 GB/sec and writing at 1.0 GB/sec. The file servers, the workstations and Windows computer are connected to our microscopes and equipment through a lab intranet with 20 Gigabit/sec network equipment, removing the network bottleneck while accessing the data. All personal and lab computers are connected to the file servers and workstations through another intranet with Gigabit/sec Ethernet equipment, making the network traffic internal.

Office:
Approximately 300 sq. ft. of private office space for Dr. Andrew Dillin and additional personnel is adjacent to the laboratory.

Environment: Next to the Dillin lab are multiple labs, which collaborate with and provide a large range of mutually beneficial scientific advances. The Tijan, Schekman, Kaufer, Shaffer, and newly hired Hockmeyer laboratories are located on the floor. Dr. Robert Tijan pioneered the identification of transcriptional networks; Dr. Randy Schekman is also located on the floor and is an expert on the transport of proteins between subcellular organelles. Dr. Rape is located one floor below and provides great insight on protein quality control in subcellular compartments. The department provides a machine shop, electronic shops, computer repair and assistance, and a chemicals and supplies storeroom. I will also be working with Dr. David Drubin, whose lab is in Barker Hall, neighboring our building; Dr. Michael Eisen, whose lab is in Koshland Hall, also neighboring our building, and Dr. Alice Ting, whose lab is in Stanford, which is accessible both through public transportation and a short drive. These laboratories all have state-of-the-art facilities and equipment as well, which all labs are willing to share for the experiments outlined in this proposal. The Meyer lab has Ph.D. staff scientists who are experts in X-ray crystallography, biochemistry, high resolution microscopy, and bioinformatics to help guide the work in this proposal.

Equipment

The Dillin lab has ownership or access to all the equipment required to perform the experiments proposed in this research fellowship application.

Media and buffers: The Dillin/Meyer lab shares a 500 sq. ft. media center for housed at Koshland Hall next door to the lab. The media facility is equipped with two thirty liter mediaclaves for automated plate pouring, multiple standard autoclaves for making of buffers and media, and all equipment required for making of other specific reagents necessary for experimentation.

Standard equipment: Within our laboratory space, we have eight full-sized incubators, five Percival incubators, three small variable incubators, two large deli cases, four full-sized -80°C freezers, three full-sized -20°C freezers, two full-sized 4°C refrigerators and a personal small 4°C refrigerator for each bench, six PCR machines, a Fischer Nanodrop 2000c, water baths, a Beckman Coulter Optima XP ultracentrifuge, a CYROPLUS 2,200 LT Store system, a transmitted light dissecting microscope for each individual researcher, a QuantStudio qPCR machine, a Seahorse XFe96 Analzyer, BioRad DNA and protein electrophoresis machinery, liquid nitrogen dewars, an Eppendorf micro-centrifuge at each benchtop, and automated gradient makers and fraction collectors. The Meyer lab houses dissecting microscopes, phosphorimager, FPLC for protein purification, analytical ultracentrifuge, real-time PCR machines, incubators and environmental chambers for nematodes, and a wide range of equipment needed for sophisticated molecular and biochemistry techniques.

Imaging: The Dillin lab owns a microscope room, which is equipped with a Zeiss AxioObserver.Z1 microscope with apotome and time-lapse imaging capabilities, a Leica DM6000 fluorescent microscope, an Echo Revolve fluorescent microscope, and three Leica M205 fully automated fluorescence Stereo Microscopes. We also have a license for Huygens Deconvolution software by Scientific Volume Imaging. We also own a large-particle COPAS Biosort for the imaging and sorting of worms, which allows detection of fluorescent intensity across large worm samples in very short periods of times. The Meyer lab owns a Leica SP8 confocal microscope, a light-sheet diSPIM microscope, a PerkinElmer spinning disk confocal, and several Zeiss microscopes equipped with motorized stages and time-lapse imaging capability. Within the Molecular Imaging Center, we have access to a large variety of confocal and multi-photon laser scanning microscopes, spinning disk confocal microscopes, lightsheet microscopes, and microscopes capable of high-resolution SIM and STORM. Together, these systems offer all the capabilities required for the proposed experiments.

Sequencing: The MCB Department houses the Vincent J. Coates Proteomics/Mass Spectrometry Laboratory and Genomics Sequencing Laboratory, as well as a Functional Genomics Laboratory, which provides services for library preparation and all Next-Generation sequencing on Illumina platforms. In addition, the Meyer lab has several Ph.D. staff scientists who are experts in bioinformatics

Computers and software: The Dillin lab is equipped with several PRISM licenses for statistical analyses, Microsoft office and adobe suites and MATLAB are offered to each scientist through UC Berkeley, every person is equipped with a work computer station (a higher-processing computer will be purchased through the K99 budget), and multiple image processing softwares are available in the lab. The Meyer lab is equipped with licenses for bowtie2, SAMtools, deepTools, macs2, bedtools, tophat, and cufflinks for post-sequencing analysis.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Ryo Middle Name Last Name*: Higuchi-Sanabria Suffix:

Position/Title*: Postdoctoral Researcher
Organization Name*: The Regents of the University of California
Department: Molecular and Cell Biology
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Applicant_Biosketch_R1.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Andrew Middle Name G Last Name*: Dillin Suffix:

Position/Title*: Professor
Organization Name*: The Regents of the University of California
Department: Molecular and Cell Biology
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Sponsor

Degree Type: PHD,BS Degree Year: 1998,1993

Attach Biographical Sketch*: File Name: D_Bio_Dillin.pdf

Attach Current & Pending Support: File Name: Current_and_Pending_Support_R1.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Barbara Middle Name J Last Name*: Meyer Suffix:

Position/Title*: Professor
Organization Name*: University of California, Berkeley
Department: Genetics, Genomics, and Develo
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Co-Sponsor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Meyer_Biosketch_R1.pdf

Attach Current & Pending Support: File Name: Current_and_Pending_Support_Meyer.pdf

APPLICANT BIOGRAPHICAL SKETCH

NAME OF APPLICANT: Higuchi-Sanabria, Ryo

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Graduate Research Assistant

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	END DATE *(or expected end date)* MM/YYYY	FIELD OF STUDY
CUNY Hunter College	B.A.	09/2006	05/2011	Biotechnology
CUNY Hunter College	M.A.	09/2006	05/2011	Biotechnology
Columbia University	M.A.	09/2011	10/2013	Nutrition and Metabolic Sciences
Columbia University	M.Phil.	09/2011	5/2014	Nutrition and Metabolic Sciences
Columbia University	PhD	09/2011	10/2015	Nutrition and Metabolic Sciences
University of California, Berkeley	Postdoctoral Fellow	01/16	--	Molecular and Cell Biology

A. Personal Statement

As a graduate student at Columbia University, my laboratory research focused on identifying novel mechanisms underlying mitochondrial and actin cytoskeletal quality control and their contributions to lifespan. I have identified a novel role for actin cytoskeletal dynamics, specifically retrograde flow of actin cables, in regulating asymmetric cell division in yeast necessary for production of an ageless daughter cell and resetting of lifespan. Subsequently, I studied the contribution of mitochondrial anchorage – mediated by mitochondrial fusion and anchorage proteins – to asymmetric cell division and lifespan regulation. In addition to these major works, I have contributed to development of methods in microscopy including pioneering a novel 3-, 4-, and 5-color live-cell imaging technique, developing super-resolution microscopy techniques, and optimization of protocols for quantification of mitochondrial quality. This all led to seven first-author papers and contributions to other work. I have also contributed to mentoring of students, laboratory management and technician duties, scientific education as a teacher's assistant and adjunct professor at Columbia University, Hunter College, and College of Mount Saint Vincent, and in clinical work shadowing a doctor of hematology, all while making exemplary progress in my thesis work. In the Dillin lab, I performed work on identifying the components of the actin cytoskeleton, which contribute to the physiological consequences of aging. Here, I found that the actin cytoskeleton declines in fitness and function during aging, and that the major transcriptional regulator, heat-shock factor-1, is required for maintenance of the cytoskeleton. In addition, I have done a significant amount of work in understanding ER quality control mechanisms, such as the unfolded protein response of the ER (UPR^{ER}) and how breakdown of these pathways contribute to aging. These studies have led to two first author papers in press, two very promising papers far along in their submission process (both manuscripts have revisions currently under review – one at *Nature* and one at *Cell*), and two other papers in or very close to submission. I believe that my productive thesis and post-doctoral work, in addition to my numerous contributions outside of the lab, provide strong evidence of my capacity to multi-task and maximize my time efficiently, making me a great candidate for the NIH Pathway to Independence Award.

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	END DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Research Aide	05/11	08/13	Biological Sciences	Hunter College	Paul Feinstein
Teacher's Assistant	09/13	12/13	Molecular Genetics	Hunter College	Paul Feinstein
Clinical Rotation	09/13	06/14	Hematology	Columbia University	Stephen Spitalnik
Teacher's Assistant	09/12	N/A	Nutrition and Metabolic Biology	Columbia University	Sekhar Ramakrishnan
Adjunct Professor	08/14	N/A	Human Genetics	College of Mount Saint Vincent	Patricia Grove/ Pamela Kerrigan

Academic and Professional Honors

IHN Training Grant 2012-2013
HHMI Med into Grad Program, Columbia University 2013-2014
TRANSFORM TL1 Award 2013-2014
Kirchstein-NRSA F32 Postdoctoral Research Fellowship 2016-2019

C. Contributions to Science

I. Made the major finding that asymmetric segregation of mitochondria has a significant impact in maintaining mitochondrial quality control and lifespan regulation. Two major methods for asymmetric segregation exists. First, **actin cytoskeleton regulates mitochondrial quality** through retrograde actin cable flow, where retrograde actin cable flow acts as a filter to prevent damaged, dysfunctional mitochondria from entering the daughter cell during yeast cell division, thus promoting production of an immaculate daughter cell with high functioning mitochondria and resetting of lifespan. Second, **mitochondrial fusion contributes to site-specific anchorage of the organelle** in the daughter cell. However, ectopically increasing anchorage is detrimental to the quality control mechanism exerted by retrograde actin cable flow. Thus, quantity and quality of mitochondrial inheritance are ensured by two opposing processes: bud tip anchorage to promote inheritance and quality control mechanisms that prevent inheritance of low functioning mitochondria.

Publications

Higuchi R, Vevea JD, Swayne TC, Chojnowski R, Hill V, Boldogh IR, and Pon LA (2013). Actin dynamics affects mitochondrial quality control and aging in budding yeast. Curr. Biol. *23*, 2417-2422. PMCID: PMC3932488. *This paper was highlighted in a "Rapid Dispatch" in Curr. Biol. 23:R107-112. It was also selected as an F1000Prime paper by the Faculty of 1000.*

Higuchi-Sanabria R, Pernice WM, Vevea JD, Alessi Wolken DM, Boldogh IR, and Pon LA (2014). Damage sequestration and organelle rejuvenation promote cellular fitness and function. FEMS Yeast. *14 (8), 1133-1146*. PMCID: PMC4270926.

Higuchi-Sanabria R, Charalel JK, Viana M, Garcia EJ, Koenigsberg A, Swayne TC, Vevea JD, Boldogh I, Rafelski S, Pon LA (2016). Mitochondrial anchorage and fusion contribute to mitochondrial inheritance and quality control in the budding yeast, *Saccharomyces cerevisiae*. MBoC. PMCID: PMC4803304.

Higuchi-Sanabria R, Vevea JD, Charalel JC, Sapar ML, Pon LA. The transcriptional repressor Sum1p counteracts Sir2p in regulation of the actin cytoskeleton, mitochondrial quality control, and replicative lifespan in *Saccharomyces Cerevisiae* (2016). Microbial Cell. PMCID: PMC5349106.

Invited Lectures

Role of actin cable dynamics in mitochondrial quality control and lifespan. 2013. Academic Retreat and 16th Wu Lectureship, Columbia University, New York, NY.

Actin dynamics affects mitochondrial quality control and aging in budding yeast. 2013 Keystone Symposia on pushing the limits of cellular quality control, Silverthorne, CO.

II. I have also made major contributions to **development of novel imaging strategies in yeast**. I have characterized novel fluorophores for utilization in 3-, 4-, and 5-color live-cell imaging using blue dyes in conjunction with teal, yellow, red, and far-red fluorescent proteins. In addition, I have optimized and **developed protocols for using fluorescent imaging to quantify mitochondrial quality and for super-resolution imaging of actin and mitochondria**.

Publications

Higuchi-Sanabria R, Swayne TC, Pon LA. Live-cell imaging of mitochondria and the actin cytoskeleton in budding yeast (2016). Methods Mol Biol. PMID: 26498779.

Higuchi-Sanabria R, Swayne TC, Boldogh IR, Pon LA (2016). Imaging of the actin cytoskeleton and mitochondria in fixed budding yeast cells. Methods Mol Biol. PMID: 26498779.

Higuchi-Sanabria R, Garcia EJ, Munteanu EL, Tomoiaga D, Feinstein P, Pon LA (2016). Characterization of fluorescent proteins for three- and four-color live-cell imaging in *S. cerevisiae*. PLoS One. PMCID: PMC4699809.

III. Provided the **first direct evidence that the actin cytoskeleton declines as a function of age** in the model organism, *C. elegans*. While many studies have implicated this fact, direct, experimental evidence has never been shown. I have qualitatively measured cytoskeletal organization as a function of age in live worms by visualizing actin cytoskeletal quality in muscle, intestine, and hypodermis during aging, and have elucidated the role of *hsf-1* in cytoskeletal regulation. In collaboration with the Herr lab, I have also **developed a novel method for biochemical quantification of F- and G-actin using a single cell western blotting platform** to interrogate cytoskeletal quality. This work also includes **identification of novel regulators of the actin cytoskeleton**.

Higuchi-Sanabria R, Frankino PA, Paul JW III, Tronnes SU, Dillin A (2018). A futile battle? Cellular quality control throughout the stress of aging. Dev. Cell. PMCID: PMC5896312.

Higuchi-Sanabria R, Paul JW III, Durieux J, Benitez C, Frankino PA, Tronnes SU, Garcia G, Daniele JR Monshietehadi S, Dillin A (2018). Spatial regulation of the actin cytoskeleton by HSF-1 during aging. Mol. Biol. Cell. PMCID: PMC6254583.

[redacted]

IV. Made the fundamental discovery that **ER stress in neurons can be communicated to distal cells through dopaminergic signaling**. Moreover, this stress signal results in massive remodeling of the ER and lipid depletion in distal tissue, which can promote increased cellular homeostasis and lifespan extension. Our findings redefine the current and historical understanding of the beneficial effects of UPR-ER acting primarily by upregulation of chaperones. In stark contrast, our findings indicate that **metabolic changes, primarily through activation of lipophagy, is a very important "non-canonical" arm of UPR-ER** that drives cellular health and lifespan. Finally, we find that lysosomal quality and function is essential in regulating ER homeostasis. Perturbations in lysosome function result in increased sensitivity to ER stress, potentially due to decreased availability of amino acids essential for glutathione synthesis. Glutathione acts in redox balance to maintain ER homeostasis. Our work **identifies the first direct link between lysosome and ER quality control through nutrient signaling via amino acids** feeding into glutathione redox handling. Finally, I have discovered a novel UPR^{ER}-independent mechanism that promotes ER quality control and lifespan through p38/MAPK pathways.

Daniele JR*, **Higuchi-Sanabria R***, Durieux J, Monshietehadi S, Ramachandran V, Tronnes SU, Kelet N, Sanchez M, Metcalf MG, Paul JW III, Gilberto G, Frankino PA, Benitez C, Zeng M, Esping DJ, Dillin A. A non-canonical UPRER promotes lipophagy to extend lifespan through dopaminergic neurons. Nature. *Revision under review*. *equal contributions *BioRxiv* **doi:** https://doi.org/10.1101/471177.

Schinzel R*, **Higuchi-Sanabria R***, Moehle EA, Webster BM, Frankino PA, Durieux J, Shalem O, Dillin A. The hyaluronidase, TMEM2, and its product regulate ER stress resistance, longevity, and pathogen resistance. Cell. *Revision under review.* *equal contributions.

[redacted]

V. Other Publications
Anderson EC, Frankino PA, **Higuchi-Sanabria R**, Qiming Y, Bian Q, Podshivolova K, Shin A, Kenyon C, Dillin A, Meyer BJ. X chromosome domain architecture regulates lifespan, but not dosage compensation. Dev. Cell. *Revision under review*.

Srivastava P, Alessi Wolken DM, Garcia-Rodriguez LJ, **Higuchi-Sanabria R**, Pon LA (2015). Organelle inheritance in yeast and other fungi. The Mycota.

Garcia EJ, de Jonge J, Stivison EA, Sing CN, Higuchi-Sanabria R, Boldogh IR, Pon LA. Reciprocal interactions between mtDNA and lifespan control in budding yeast. MBoC. *Under revision*.

D. Additional Information: Research Support

Ongoing Support

F32 AG 053023 (Higuchi-Sanabria, Postdoctoral Fellow) 07/01/2016-06/30/2019
NIH The Cytoskeletal Stress Response: A Novel Facet to Protecting Cell integrity During Aging.
This proposal is aimed at identifying a novel cellular stress response dedicated to conserving the function of the cytoskeleton under conditions of stress and advanced aging and will contribute to identification of novel targets for therapeutic interventions in age-related disease.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Andrew Dillin

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: 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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
UNIVERSITY OF NEVADA	BS	09/1989	09/1993	BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA	PHD	07/1993	07/1998	GENETICS
UCSF – POST-DOC	NA	09/1998	09/2002	GENETICS

A. Personal Statement

My lab focuses on the questions of why an aging organism begins to lose control over the integrity of its proteome, and how this loss is communicated across its various tissues. We have developed and applied techniques that allow us to manipulate signaling pathways or proteins within a single tissue, cell or an organelle within a single cell so that we can observe how a small perturbation might reverberate and effect the physiology of the whole organism. Our research has shown that the stress response pathways that ensure proteostasis are not restricted to cell autonomous events, but can be communicated across an organism. We have reported that this is found for the unfolded protein response of the mitochondria (UPR^{MT}), and now find distinct signaling events for the UPR of the endoplasmic reticulum (UPR^{ER}) as well as the cytosolic heat shock response (HSR). Collectively, we have discovered at least three distinct signaling events that communicate cell non-autonomously to effect organismal stress responses and aging. Our research strongly suggests that intracellular and even organelle-specific stress can be communicated extracellularly via a regulated, endocrine-like process to impinge upon the aging process and health.

My lab right now is absolutely committed to understanding ER proteostasis and aging, with four post-doctoral fellows and two graduate students working diligently this area of biology. My lab is also poised to comprehensively dissect cell-autonomous and cell-nonautonomous stress communication mechanisms within and across cells in multiple model organisms. As a group leader, I have always encouraged my postdocs and graduate students to explore new areas of biology and reach out of their collective comfort zone. Ashley Frakes' background in neurodegeneration and career goals will lend well to my training strategy, which will allow her to grow in this new area of research. This approach has allowed my laboratory to make important advances in many areas, including mitochondrial biology, protein homeostasis, insulin/IGF1 signaling, and ER stress. In summary, I have demonstrated both a strong record of creative scientific achievements and preparing students and postdocs for the next level of their respective careers. We strive to use every useful biological tool available to us, and when the expertise is not available within the lab, we actively collaborate to ensure efficient progress forward.

Berendzen KM, Durieux J, Shao LW, Tian Y, Kim HE, Wolff S, Liu Y, Dillin A. Neuroendocrine Coordination of Mitochondrial Stress Signaling and Proteostasis. Cell. 2016 Sep 8;166(6):1553-1563. PMID: 27610575

Kim HE, Grant AR, Simic MS, Kohnz RA, Nomura DK, Durieux J, Riera CE, Sanchez M, Kapernick E, Wolff S, Dillin A. Lipid Biosynthesis Coordinates a Mitochondrial-to-Cytosolic Stress Response. Cell. 2016 Sep 8;166(6):1539-1552. PMID: 27610574

Tian Y, Garcia G, Bian Q, Steffen KK, Joe L, Wolff S, Meyer BJ, Dillin A. Mitochondrial Stress Induces Chromatin Reorganization to Promote Longevity and UPR(mt). Cell. 2016 May 19;165(5):1197-208. PMID: 27133166

Durieux, J. Wolff, S. & Dillin A. (2011). The cell non-autonomous nature of electron transport chain-mediated longevity. Cell, 144(1), 79-91. PMCID: PMC3062502.

Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C. Rates of behavior and aging specified by mitochondrial function during development. Science. 2002;298(5602):2398-401. PMID: 12471266.

B. Positions and Honors

Positions and Employment

1990-1991 Undergraduate Student with Dr. Jeff Seemann, University of Nevada. Regulation of RUBISCO in the spinach plant.
1991-1993 Undergraduate Student with Dr. Ardythe McCracken, University of Nevada. ER associated protein degradation.
1993-1998 Graduate Student with Dr. Jasper Rine, University of California, Berkeley. Studies of transcriptional repression, regulation of replication initiation and mitosis in yeast.
1998-2002 Postdoctoral Fellow with Dr. Cynthia Kenyon, University of California, San Francisco, Determinants of longevity in the nematode *Caenorhabditis elegans.*
2002-2007 Assistant Professor, The Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory La Jolla, CA
2007-2011 Associate Professor, The Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory, La Jolla, CA
2007-2012 Adjunct Associate Professor, University of California, San Diego, CA
2008-present Investigator, Howard Hughes Medical Institute
2009-2012 Director, Glenn Center for Aging Research at the Salk Institute
2011-2012 Professor, The Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory, La Jolla, CA
2012-present Professor, University of California at Berkeley, Molecular and Cell Biology Department

C. Contributions to Science

1. Cell non-autonomous communication can influence stress responses and longevity. My lab made the fundamental discovery that protein stress in one cell's organelles can be communicated to distal cells yet to undergo proteotoxic stress. We have shown that unfolded protein stress in neurons can be communicated to distal cells, resulting in upregulation of the stress responsive UPR^{ER}. That these distal tissues can upregulate transcriptional programing that alters organismal aging was surprising and has encouraged us to delve deeper into the mechanism of transcellular signalling. In addition to this ER cell non-autonomous research, the Dillin Lab previously discovered a similar system for the communication of stress within the mitochondria and by cytoplasmic heat shock transcription factor HSF-1.
   - Taylor, R. & Dillin, A. (2013). XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell,153(7), 1435-47. PMCID: PMC4771415.
   - Douglas, P, Baird N, Simic M, Uhlein S, McCormick M, Kennedy B, Dillin A. Heterotypic Signals from Neural HSF-1 Separate Thermotolerance from Logevity. Cell Reports 12, 1196–1204 August 18, 2015. PMID: 26257177

2. The first report demonstrating that targeting a longevity pathway can combat an age related neurodegenerative disease. While our lab first reported the effects of IGF-1 signalling on Aβ toxicity in worms, we later demonstrated that these findings were conserved in a mouse model for Alzheimer's Disease (AD), opening up novel pathways that can be targeted by therapeutics in an attempt to treat this devastating disease. This work also provided the first experimental evidence that formation of Aβ plaques from toxic Aβ oligomers is a protective event along the course of Alzheimer's Disease progression. This body of work has become well accepted by nearly all of those who study protein-misfolding diseases. We

have expanded these findings to show that HSF-1 plays multiple roles beyond just the upregulation of chaperones, and instead works to protect the cell by mediating cytoskeletal health during aging.

- Cohen, E., Bieschke, J., Perciavalle, R., M., Kelly, J. W. & Dillin, A. (2006). Opposing activities protect against age-onset proteotoxicity. Science, 313(5793),1604-1610. PMID: 16902091

- Cohen, E. Paulsson, J.F., Blinder, P., Burstyn-Cohen, T., Du, P., Estepa, G., Adame, A., Pham, H.M., Holzenberger, M., Kelly, J.W., Masliah, E. & Dillin, A. (2009). Reduced IGF-1 Signaling Delays Age Associated Proteotoxicity in Mice. Cell, 139(6),1157-69. PMCID: PMC3017511.

- Baird NA, Douglas PM, Simic MS, Grant AR, Moresco JJ, Wolff SC, Yates JR 3rd, Manning G, Dillin A.(2014). HSF-1-mediated cytoskeletal integrity determines thermotolerance and life span. Science, 346(6207), 360-3. PMID: 25324391

3. Identification of the first factor essential and specific for diet restriction induced longevity. Since the first discovery of diet restriction mediated longevity reported by McCay in 1929, the aging field has long searched for the underlying genetic requirements of this process. In our Nature papers, we reported and characterized conserved components of the genetic pathway required for this phenomenon. Our lab is thus credited with the discovery of the genetics behind the diet restriction pathway. The pathway we discovered in worms has subsequently been shown to play a role in the regulation of glucose homeostasis, insulin secretion, hepatic triglyceride synthesis, fatty acid transport, and IGF-1 signalling in adult mammalian cells.
   - Panowski, S., Wolff, S., Aguilaniu, H. & Dillin, A. (2007). PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. Nature, 447(7144), 550-556. PMID: 17476212

   - Mair, W., Morantte, I., Rodrigues, A.P.C., Manning, G., Montminy, M., Shaw, RJ., Dillin, A. (2011) CRTC-1 couples energy homeostasis to longevity. Nature, Feb; 470(7334):404-8. PMCID:21331044.

4. First identified that mice lacking TRPV1 pain receptors are long-lived, displaying a youthful metabolic profile at old age. We then found evidence that levels of the secreted peptide CGRP were negatively associated with metabolic health during aging. These data suggest that ablation of select pain sensory receptors or the inhibition of CGRP is associated with increased metabolic health and control longevity.

   - Riera CE, Huising MO, Follett P, Leblanc M, Halloran J, Van Andel R, de Magalhaes Filho CD, Merkwirth C, Dillin A. (2014) TRPV1 pain receptors regulate longevity and metabolism by neuropeptide signaling. Cell. 2014 May 22;157(5):1023-36. PMID: 24855942

5. Made the discovery that one of the by-products of longevity is an increased proteasome activity and “stemness” in somatic cells upon a block in reproduction. The somatic cells of worms without a germline are capable of mounting an enhanced defence against proteotoxic stressors such as those found in Huntington’s Disease. Mammalian embryonic stem cells also exhibit a similar heightened level of proteostasis that is determined by the activity of its proteasome.
   - Vilchez D, Morantte I, Liu Z, Douglas PM, Merkwirth C, Rodrigues AP, Manning G, & Dillin A. (2012). RPN-6 determines C. elegans longevity under proteotoxic stress conditions. Nature, 489(7415), 263-8. PMID: 22922647

   - Vilchez D, Boyer L, Morantte I, Lutz, M, Merkwirth C, Joyce D, Spencer B, Page L, Masliah E, Berggren WT, Gage FH, and Dillin A. Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. Nature. 2012: 489(7415):304-8. PMID: 22972301.

D. Additional Information: Research Support

Ongoing Research Support

1R01AG059566-01 (Dillin, PI) 07/15/2018-03/31/2023
NIH/NIA
Glial regulation of longevity through a transcellular unfolded protein response.
The major goals of this project are to elucidate what differentiates glial vs. neuronal cell non-autonomous UPR^{ER} and to uncover the mechanisms by which peripheral tissues sense UPR^{ER} signals from glia.
*The aims in this grant are unique from Ashley’s K99/R00 application and there is no overlap.

R01ES021667 (Dillin, PI) 03/01/2012-06/30/2022
NIH
Distal Mitochondrial Signaling in a Multicellular Organism
The major goal of this project is to perform research on how mitochondrial stress in one cell type can be communicated to a distal cell type. Role: PI

R01AG055891 (Dillin, PI) 04/01/2017-03/31/2022
NIH/NIA
The Collapse of Proteostasis during Aging is Mediated by Cytoskeletal Actin Functions
The major goal of this project is to perform research towards the cytoskeleton's role in maintaining the overall health of the cell. Role: PI

R37AG024365 (Dillin, PI) 09/01/2004-03/31/2021
NIH/NIA
The Perception of Mitochondrial Stress in Receiving Cells
The major goal of this project is to determine how distal tissues can sense mitochondrial stress in other tissues, and how their own form and function might change in response to distal mitochondrial signaling. Role: PI

R01AG042679 (Dillin, PI) 03/15/2013-02/28/2018
NIH/NIA
The Cell Non-Autonomous Nature of UPR Signaling
The major goal of this project is to discover how the UPR within the endoplasmic reticulum with neurons can communicate with distal tissues to increase the chance of survivorship as the organism ages. Role: PI

-- (Dillin, PI) 09/01/2008-08/31/2024
Howard Hughes Medical Institute
Molecular Pathways of Aging
The major goal of this project is to perform high risk, innovative research towards the understanding of aging and age-related diseases. Role: PI

Completed Research Support

R01AG042679 (Dillin, PI) 03/15/2013-02/28/2018
NIH/NIA
The Cell Non-Autonomous Nature of UPR Signaling
The major goal of this project is to discover how the UPR within the endoplasmic reticulum with neurons can communicate with distal tissues to increase the chance of survivorship as the organism ages. Role: PI

RB5-06974 (Dillin, PI) 03/01/2014-02/28/2017
California Institute for Regenerative Medicine
A Requirement for Protein Homeostasis in the Mediation of Stem Cell Health
The major goal of this project is to understand the behaviors and regulation of UPR and stress responses in stem cells. Role: PI

R01 ES021667 (Dillin, PI) 10/19/2012-12/31/2016
NIH/NIA
Distal Mitochondrial Signaling in a Multicellular Organism
The major goal of this project is to discover how mitochondria within the nervous system can communicate a signal that will ensure the survival of an animal under conditions of stress. Role: PI

R01 AG027463 (Dillin, PI) 07/01/2008-06/30/2014
NIH/NIA
Genetic Regulation of the Response to Dietary Restriction
The major goal of this project is to understand the molecular mechanism by which a core-signaling pathway that responds to and integrates an organism's response to reduced caloric intake, perceives and interprets the environmental signals that ultimately result in increased longevity. [Funds shared by two investigators.] Role: PI

P01 AG031097 (Kelly, PI) 02/01/2009-01/31/2014
NIH/NIA
Molecular Mechanisms Linking Aging, Abeta Proteotoxicity, and Neurodegeneration
The major goal of Project 3, Age-Associated Neuroprotection by Insulin/IGF-1 Signaling: From Worm to Mouse, is to investigate the molecular mechanisms that prevent proteotoxicity during early life that become compromised with age. Role: PI

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: **Barbara J. Meyer**

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: **Professor of Genetics, Genomics, and Development at U. C. Berkeley; Investigator of the Howard Hughes Medical Institute**

EDUCATION/TRAINING *(Begin with baccalaureate or professional education, include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	COMPLETION	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

A. Personal Statement

[redacted]

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Ryo		Higuchi-Sanabria		PD/PI		12.0			75,000.00	12,750.00	87,750.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **87,750.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**87,750.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,000.00
2. Publication Costs	4,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**19,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**112,750.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	8.0	112,750.00	9,020.00
		Total Indirect Costs	**9,020.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Cost Allocation Services - Arif M. Karim - 415/437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**121,770.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	121,770.00

L. Budget Justification* File Name: D_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Ryo		Higuchi-Sanabria		PD/PI		12.0			75,000.00	12,750.00	87,750.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **87,750.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**87,750.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 2**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,000.00
2. Publication Costs	4,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**19,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**112,750.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	8.0	112,750.00	9,020.00
		Total Indirect Costs	**9,020.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Cost Allocation Services - Arif M. Karim - 415/437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**121,770.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	121,770.00

L. Budget Justification* File Name: D_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Ryo		Higuchi-Sanabria		PD/PI		12.0			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 3**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . R00 Phase	249,000.00
Total Other Direct Costs	**249,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**249,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**249,000.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	249,000.00

L. Budget Justification*	File Name: D_Budget_Justification.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Ryo		Higuchi-Sanabria		PD/PI		12.0			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 4**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . R00 Phase	249,000.00
Total Other Direct Costs	**249,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**249,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**249,000.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	249,000.00

L. Budget Justification*	File Name: D_Budget_Justification.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Ryo		Higuchi-Sanabria		PD/PI		12.0			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 5**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . R00 Phase	249,000.00
Total Other Direct Costs	**249,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**249,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**249,000.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	249,000.00

L. Budget Justification*	File Name: D_Budget_Justification.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

Mentored K99 Phase:

Personnel: Ryo Higuchi-Sanabria, Ph.D., postdoctoral fellow is requesting two years of funding for the K99 mentored portion of the grant with a proposed salary of $75,000 per year, based on National Institute of Aging guidelines.

Dr. Andrew Dillin will serve as the mentor for the proposed research project and is available as needed throughout the duration of the mentored K99 phase. No salary is requested for the mentor.

Travel: A travel budget of $6,000 per year is requested to be used towards conferences in *C. elegans* aging, stress response, cytoskeletal biology, and courses on bioinformatics and proteomics (planned two conferences/year). For the planned two conferences, $1500 will be used on registration, $2000 will be used on flights and travel, and $2500 will be used on hotels and meals each year.

Supplies: Project-related expenses for the training aspects of the project are estimated at a total of $15,000/year. These expenses will include laboratory supplies, textbooks, fees associated with UC Berkeley Illumina Sequencing, fees associated with UC Davis proteomic sequencing, and a high-processing power computer to be used for image and bioinformatics analyses.

Publication costs: One larger manuscript and one smaller methods manuscript is predicted to be published within the mentored K99 phase, which we request $4000 to offset costs. All other manuscripts are planned to be published during the R00 phase.

Indirect costs are calculated at 8% of the Modified Total Direct Cost base rate, and has been applied to the budget.

Independent R00 Phase:

The three years of the R00 phase requests a budget of $249,000/year, and a specific budget justification will be provided upon receipt of an independent faculty position.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		175,500.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		175,500.00
Section C, Equipment		0.00
Section D, Travel		12,000.00
1. Domestic	12,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		785,000.00
1. Materials and Supplies	30,000.00	
2. Publication Costs	8,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	747,000.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		972,500.00
Section H, Indirect Costs		18,040.00
Section I, Total Direct and Indirect Costs (G + H)		990,540.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		990,540.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12892985 Funding Opportunity Number: PA-19-130 . Received Date: 2019-07-01T16:26:57.000-04:00

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	Introduction_Letter_R1.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	Candidate_Background_R1.pdf
Research Plan Section	
3. Specific Aims	Specific_Aims_R2.pdf
4. Research Strategy*	Research_Strategy_R1.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	D_Responsible_Conduct.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	Sponsor_Information_R1.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	Letters_of_Support_R1.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	Institutional_Environment_R1.pdf
11. Institutional Commitment to Candidate's Research Career Development	F_Institutional_Committment.pdf
Other Research Plan Section	
12. Vertebrate Animals	
13. Select Agent Research	
14. Consortium/Contractual Arrangements	
15. Resource Sharing	F_Resource_Sharing.pdf
16. Authentication of Key Biological and/or Chemical Resources	D_Key_Resources.pdf
Appendix	
17. Appendix	

Funding Opportunity Number: PA-19-130. Received Date: 2019-07-01T16:26:57.000-04:00

PHS 398 Career Development Award Supplemental Form

Citizenship*:

18. U.S. Citizen or Non-Citizen National?* [redacted]

If no, select most appropriate Non-U.S. Citizen option

[redacted]

If you are a non-U.S. citizen with a temporary visa applying for an award that requires permanent residency status, and expect to be granted a permanent resident visa by the start date of the award, check here: [redacted]

Overall, the tone of the reviewers was very positive. They have unanimously rated highly both myself as a candidate and Dr. Andrew Dillin as a sponsor for the proposed work. In addition, the reviewers have shown high interest in the research proposal, its innovation, and its potential seminal discoveries in their contributions to the biology of the cytoskeleton and aging. The reviewers' enthusiasm was dampened by a weak career development/training plan, as well as the lack of experimental detail, statistics, and caveats in the proposed research plan. I have made considerable effort in addressing each of these issues as highlighted below:

Recommendations for Career Development and Training Plan:

1) Lack of proposed formal training in aging, incorporate aging into future work, put a biology of aging faculty on advisory committee, and increase connection to Glenn Foundation Center. *While my graduate work focused in yeast biology, I studied the contribution of asymmetric cell division to lifespan and regulation of aging. The research background was edited to explicitly state this training. Moreover, the training plan was extensively updated to incorporate a section on aging, including numerous workshops and meetings in the aging field, as well as in-depth involvement in the Glenn Foundation: 1) Anne Brunet, world-renowned aging scientist and Glenn Foundation director, was added as an advisor, 2) Roberto Zoncu, a recipient of the Glenn Foundation Award, who is a collaborator of our lab has included a reference letter, 3) I will present my work at the Bay Area Aging Meeting sponsored by Glenn Foundation in 2020 and participate in other Glenn Foundation events.*

2) Plan for supervision/mentoring of candidate is weak. *Both the sponsor letter and training sections have been significantly strengthened: 1) Barbara Meyer has been added as a co-sponsor to increase mentorship and training in chromatin biology and bioinformatics. 2) Additional advisors have been added: Anne Brunet as an aging biologist and Amy Herr as a bio-engineer. 3) The section describing the role of each mentor has been greatly expanded, and a table specifying what areas of expertise each mentor will provide, as well as how involved each mentor will be for each specific sub-aim has been added. 4) Each mentor has been given a specific amount of dedicated time for training and mentorship directly in their labs, and specific students/postdocs in each lab have been assigned as trainers from each collaborator and advisor (see letters of support and sponsor letter).*

3) Career development is largely what the candidate is already doing. *All sections of what I have already received exemplary training (writing, management, teaching, increasing diversity, etc.) have been significantly shortened to make space for more significant career developments: increasing skills in biochemistry, mass spectrometry, bioinformatics, and creating a niche in aging biology, with specific courses, workshops, and meetings included.*

Recommendations for Research Proposal:

1) Lack of experimental details: statistics, number of animals, number of trials. *Statistics, number of animals, and number of replicates has been added to each aim (excepting those that are repetitive: e.g. lifespan stats are only included on the first mention of lifespans. All experimental details and statistical measurements are italicized.*).

2) Overly ambitious proposal that would require too much work. Should remove neuronal *hsf-1* section to make the proposal more cohesive. *Entire aim on studying neuronal hsf-1 has been removed, and proposal has been trimmed significantly, while still incorporating the suggested additions. Now there are only 2 aims that very closely complement each other. Finally, while the proposal is certainly ambitious, it has been clearly stated by all references, collaborators, sponsors, and myself that I am a very dedicated and hard-working scientist whose past productivity suggests that this proposal can be done. Moreover, we have 2 sponsors and 5 collaborators/advisors on the panel, as well as two additional collaborators (one who has written a reference), all of whom will contribute greatly to the mentorship and success of this work. Thus, with this powerful team, we believe that this highly ambitious proposal can be achieved in the grant's period.*

3) Increase the number of caveats and pitfalls. *Every section now contains at least 1 (2 in some cases) caveats that offer alternative hypotheses, alternative experiments, or alternative methods. The caveats have also been expanded to provide more details. All caveats in each section are highlighted with underlining.*

4) Relationship to aging of the aims is questionable, especially of the impact of aging on chromatin modifiers. *The entire proposal is built on the hypothesis that cytoskeletal breakdown is what ultimately leads to the physiological consequences of aging. Our previous work have shown that actin exhibits functional breakdown during aging. Moreover, preventing this extends lifespan, while premature actin breakdown decreases lifespan. Thus, a proposal aimed at identifying novel components that preserve actin is certainly an aging proposal as it nests on the hypothesis that protecting actin (by manipulating the pathways identified here) can extend lifespan and promote healthy aging. While this proposal primarily focuses on understanding what causes the functional breakdown of actin through the aging process, this is essential to the study of cytoskeletal aging. This has been made explicitly clear throughout the proposal. Moreover, almost every aim has been given an aging component (either measuring actin cytoskeletal integrity during aging, impact of manipulating genes on lifespan, studying the form and function of proposed mechanisms throughout the aging process, etc.). There is also now a sub-aim proposing to study the function of chromatin modifiers and chromatin state during the aging process.*

Candidate Background. I believe that I harbor a unique and eclectic skillset gained through years of devoted time both inside and outside of the laboratory. Countless hours in restaurant management have taught me invaluable lessons like the ability to husband resources and impeccable time management, while my teaching experience has granted me articulate communication, mentoring capacity, and leadership. These worldly experiences combined with my expertise on the actin cytoskeleton, cellular stress response, and aging, create an idiosyncratic profile, which I believe makes me a great candidate for the NIH Pathway to Independence award.

At Columbia, I worked vigorously in researching aging using the yeast model, *S. cerevisiae*. Within a year, I put together a manuscript that highlighted the importance of the actin cytoskeleton and its role in mitochondrial quality during aging, *a work that was published in Current Biology and featured as an F1000 prime article.* My dedication has led to *seven first-author publications in the three years spent in the lab*. My experiences have made me resilient and efficient, allowing me to *complete my PhD within four years with a 4.0 GPA*. In the Dillin lab, I am continuing my work on aging in the context of cellular stress response. I focused on two broad subjects: the breakdown of the cytoskeleton during aging, which my proposal aims to explore further, and the unfolded protein response of the endoplasmic reticulum (UPR^{ER}) and its impact on cellular metabolism and aging. I have created tools to interrogate cytoskeletal quality during aging in an effort to answer open questions that have been unanswered for decades due to the lack of tools to interrogate the cytoskeleton. This work has been *published in the Molecular Biology of the Cell, along with a comprehensive review on cellular stress responses*. I have also built many fruitful and mutually beneficial collaborations both inside and outside of Berkeley, including collaborations with Dr. Barbara Meyer, Dr. Roberto Zoncu, and Dr. Amy Herr at UCB and with Dr. Valerie Weaver at UCSF. Several of these works are expected to be in press by the end of 2019 or early 2020.

In the ER field, I have worked with members of the Dillin lab to develop independent projects on ER quality control. First, I have found that ER stress can be communicated from neurons to activate UPR^{ER} signaling in distal tissue, partially through dopaminergic neurons. Moreover, this signal activates a unique and non-canonical arm of UPR^{ER} involved in ER remodeling and activation of lipophagy, which benefits lifespan. This work has been favorably reviewed at *Nature* and the revision is currently under review. I have also discovered a novel communication from the lysosome to the ER through amino acids, which is required for proper ER quality control through UPR^{ER} activation. This work is being prepared for submission to *Nature Metabolism*. Finally, I have discovered a novel UPR^{ER}-independent mechanism that promotes ER quality control and lifespan through p38/MAPK pathways. This work has been favorably reviewed at *Cell*, and the revision is currently under review.

I believe that my comprehensive work in multiple fields highlights my broad range of interests and ability to succeed in many different situations. My numerous collaborations show that I am capable of working well with others, building meaningful relationships, and multi-tasking. I am confident that my educational, worldly, and scientific experience illustrates my capacity to succeed through the proposed training to complete my postdoctoral work and transition into a tenure-track faculty position. With my work ethic, experience, and the knowledge of my committee, I have the tools to succeed in this ambitious, but highly important research proposal.

Career Goals and Objectives. My short term research goal is to continue pursuing my interests in interrogating the impact of actin cytoskeletal health on the physiological consequences of aging. I hope to establish an independent research group focusing on dissecting the complex map involved in cytoskeletal regulation. Obtaining the Pathway to Independence Award will give me the time to acquire new skills and knowledge, particularly in data science to dissect large sequencing and proteomic datasets with the help of Drs. Barbara Meyer, Alice Ting, Brett Phinney, and Michael Eisen. I will also expand my skills in biochemistry with the help of Drs. David Drubin and Amy Herr. These skillsets will be invaluable in my progression to a faculty position, where I will combine these tools with my expertise in cell biology and genetics to map cytoskeletal regulation and its breakdown during the aging process. My mentor, Dr. Andrew Dillin, and advisor, Dr. Anne Brunet, will play a pivotal role in providing training and mentorship in aging biology to help create my niche in this growing field.

Long term, I plan to expand my work to the cross communication of cytoskeletal stress response to the quality control and fitness of other organelles. My proposal touches on this briefly in an effort to study the communication between lipid homeostasis and cytoskeletal regulation, but in future proposals, I hope to interrogate the impact of cytoskeletal quality and health to other organelles, including ER and the mitochondria, both of which I have developed expertise in studying throughout my scientific career. All of these studies will be performed in the context of aging. In my previous research, I have shown that many organelles: the cytoskeleton, ER, mitochondria, and lipid droplets, are all dysregulated and experience functional breakdown during the aging process. However, much of my research has been focused on studying these significant cellular components independently. Similarly, most aging groups study these organelles independently and there are few studies which aim to study interorganelle communication throughout the aging process. The training plan proposed here will give me the tools and expertise to answer these significant questions in the field of aging biology.

I hope to develop my research group in an academic environment with passion, dedication, and respect towards teaching and mentorship, such as in State University systems. I have taught courses at Columbia and Hunter college, have been an adjunct professor at the College of Mount Saint Vincent, and taught and mentored many high school, undergraduate, masters, and graduate students in and out of the lab, which I believe make me proficient in teaching/mentoring. I am also highly invested in increasing the presence of under-represented minority in STEM, and have been involved directly in programs focused in this cause including the NIH Bridges to Baccalaureate (B2B) program and the CHORI Summer Research program. I hope to evolve programs similar to these in my research institute to continue to expand science to a larger population of racial and socioeconomic minorities. I truly believe that an individual who can succeed in higher education while maintaining families and multiple part-time and full-time obligations is one who is sure to succeed in scientific research, and we cannot continue to lose these brilliant minds to other fields, simply due to lack of opportunities open to them. While the primary criticism of my interest in teaching may be to question my dedication for research, I combat this criticism by expressing my profound belief that teaching and education are the essence of research. To adequately teach and educate the next generation is to both preserve and expand the excellence of scientific research. It is through the scientists of tomorrow that we can make research an eternally successful creature.

Independence from Mentor: Dr. Dillin has an incredible reputation for being a supportive mentor and pushing his trainees to transition to independence. As evidenced by his previous postdoctoral trainees who have established their own labs, he maintains open communications, open availability of strains and resources, and complete transparency to avoid scientific overlap. Moreover, my work on cytoskeletal biology is unique in the Dillin lab, as this is not a primary focus of interest to Dr. Dillin or other members of the lab, so I do not anticipate heavy overlap. Dr. Dillin has also assured me that I am free to take any reagents and strains I need to establish my own lab, and that the scientific discoveries I make during the K99 phase will intellectually belong to me.

Plan for Career Development/Training Activities during Award Period. Throughout the award period, I will direct a majority of my time to the proposed research. My previous scientific training has been focused on understanding cell biology and genetics. This additional training will expand my experimental skillsets to data science, including understanding large transcriptome, ChIP-seq, ATAC-seq, and proteome datasets. The scientific training and mentorship I receive will be focused on aging, cytoskeleton, and chromatin biology.

Learn more about biochemistry/mass spectrometry: my training in biochemistry is limited, and I plan to address this issue during the K99 award period by working closely with the Ting lab to develop an assay for proximity labeling of molecular interactors of actin. I will educate myself by reading instructional text, such as *Mass Spectrometry for Biotechnology* by Gary Suizdak, and I will also take instructional lessons at the Proteomic core at UC Davis. Moreover, I will continue to work closely with the Herr lab in learning how to biochemically interrogate cytoskeletal fitness and function, and will attend a Single Cell Analysis workshop run every summer at the Cold Spring Harbor, which provides cutting-edge technologies for characterization of single cells. Finally, I will continue collaborations with the Zoncu lab to incorporate more biochemical tools into my experimental pipeline. I am currently working with Dr. Roberto Zoncu in characterizing the impact of lysosomal function on ER quality control, but we have a mutually shared interest to continue collaborations into other projects.

Applying bioinformatics techniques: As a cell biologist and geneticist, my skillsets in bioinformatics are basic. Thus, I will spend a large amount of time with a post-doc in the lab, Dr. Raz Bar-Ziv, who is an expert in bioinformatics techniques using Galaxy and Matlab. I have already begun training with him in understanding how to analyze transcriptome datasets, and will continue to expand my knowledge by training with him and members of the Eisen and Meyer lab to learn how to apply these techniques to other large datasets, including ChIP-seq and ATAC-seq. I also plan to take *Databases and tools of Bioinformatics* offered by the UC Berkeley learning center and the UC Berkeley “Become a Web Developer in 24 Weeks” coding bootcamp.

Creating a niche in aging biology: In the Pon lab, I worked intensely in the aging field, studying the impact of asymmetric cell division on yeast lifespan. In the Dillin lab, I use primarily *C. elegans* to study the impact of stress in aging, and use human cell culture to model the conservation of my findings in a mammalian system. I will continue to expand my experimental and scientific techniques and knowledge on organismal and human aging with the help of the many *C. elegans* experts in the Dillin lab, including Drs. Jenni Durieux and Joe Daniele, and human cell culture experts, Drs. Erica Moehle and Robert Schinzel. I already collaborate with these, and many other, bright scientists in the lab, and have created mutually beneficial relationships to expand my skillsets and produce high-quality manuscripts. Moving forward, I will also work together with leading aging scientist, Dr. Anne Brunet, and her lab to maximize my training in aging. Dr. Brunet plays an integral role in the Glenn Foundation and organizes the Bay Area Aging Meeting, and therefore will be a phenomenal resource not only experimentally and scientifically, but to help create a niche for my own research group in the expanding aging field. To further develop my involvement in the Glenn Foundation and the general aging community, I participate annually in the

Bay Area Aging meeting, plan to attend the Paul F. Glenn Symposium for the Biology of Aging at Harvard in May 2019, will attend the monthly Glenn Center seminar series titled "Frontiers in Aging," have added Dr. Anne Brunet to my advisory committee, and will continue to collaborate with Glenn Foundation award recipient, Dr. Roberto Zoncu on an exciting project in lysosomal biology. I also plan to attend the National Institute of Aging Summer Training Course in 2020. Finally, I plan to attend at least a few of the following seminars in aging biology: American Society on Aging America conference series, GSA Annual Scientific Meeting, Molecular Biology of Ageing Meeting, Keystone Mitochondria in Aging and Age-Related Disease, bi-annual Aging, Metabolism, Stress & Pathogenesis and Small RNAs in *C. elegans* at Madison, and International Symposium on Healthy Ageing.

Advisory Committee: I will expand my experimental skills while establishing my fundamental work in cytoskeletal biology in the aging field. Moreover, I will gain new collaborations and career training from diverse experts to increase my scientific skills and my capacity to serve as a principal investigator. I have designed a scientific advisory panel including experts in diverse fields within the San Francisco Bay Area. I will work directly with the members of these labs, and have committee meetings every 6-9 months during the K99 phase. We will work on my scientific progress, career development, and future publication and grant applications.

Transcription expert: Michael Eisen, Ph.D. is a very well-known HHMI scientist whose lab applies a diverse array of biochemical, genetic, and computational approaches to answer complex questions about enhancers and general chromatin state. His expertise will be critical as I pursue complex techniques requiring both experimental and computational skills, such as ChIP-seq and ATAC-seq. Together with co-mentor, Dr. Barbara Meyer, the Eisen lab will provide a critical framework for my training in interrogating chromatin structure using bioinformatics tools. In depth in-laboratory training will occur in both labs: I will spend a full month in the Eisen lab working with Drs. Xiao-Yong Li and Colleen Hannon to learn how to perform and analyze ChIP-seq and ATAC-seq.

Cytoskeleton expert: David Drubin, Ph.D. is the co-chair of the MCB department at Berkeley and Ernette Comby Chair in Microbiology. He is a renowned expert on cytoskeletal biology, using both real-time microscopy of live cells and *in vitro* techniques to interrogate the cytoskeleton and its many functional roles. I will participate in all Drubin lab meetings and present my own data every 3-6 months to get direct feedback on my work involving the cytoskeleton. I will also meet with Dr. Drubin every month to discuss research and career progression, and will work directly with graduate student, Bob Cail, as my primary point person for day-to-day guidance.

Proximity labeling expert: Alice Ting, Ph.D. is a Chan Zuckerberg Biohub investigator and a professor at Stanford University. Her lab has truly revolutionized the field of proximity labeling and developed the miniTurbo method. I will train in the Ting lab for 3-6 weeks to troubleshoot and develop a miniTurbo method for *C. elegans*.

Aging expert: Anne Brunet, Ph.D. is a world-renowned investigator in aging biology, whose lab has pioneered the African killifish as a new model to explore regulation of aging and age-related diseases. The Brunet lab also specializes in understanding the epigenetics of aging through changes in chromatin structure in *C. elegans* and human cells, providing the perfect expertise for my proposal. I will meet with Dr. Brunet every 2-3 months, and present in her lab meeting for critical feedback at least every 6 months. Moreover, I will work closely with Dr. Brunet in establishing a closer relationship with the Glenn Foundation, specifically by taking active participation in organizing the Bay Area Aging Meeting and other events held at Stanford, UCSF, UCB, and Buck campuses.

Bio-engineering expert: Amy Herr, Ph.D. is a Chan Zuckerberg Biohub investigator and professor at UC Berkeley who is the leader in single-cell immunoblot tools. I am working closely with the Herr lab to create a biochemical method to quantitatively assess actin function with single cell resolution. I engage with Dr. Herr and her lab on a regular basis, and will continue to be involved directly in the work, participate in lab meetings, and meet with Dr. Herr at least every 3-6 months to expand my skills and knowledge in biochemistry and bio-engineering.

Involvement of Mentoring Committee

Role	Anne Brunet	David Drubin	Mike Eisen	Amy Herr	Barbara Meyer	Alice Ting
Career Training & Scientific Guidance	*aging; chromatin; C. elegans; cell culture*	*cytoskeleton; cell culture; cell biology*	*chromatin; bioinformatics; cell biology*	*scWB; biochemistry*	*chromatin; bioinformatics; C. elegans*	*proximity labeling; biochemistry; proteomics*
Aim 1.1	*monthly meetings*	*meet ~2-3 months*	*full month training*		*continuous training*	
Aim 1.2	*monthly meetings*	*meet ~2-3 months*	*meet ~2-3 months*		*continuous training*	
Aim 2.1		*meet ~2-3 months*			*monthly meetings*	*3-6 week training*
Aim 2.2	*meet ~3-6 months*	*meet ~2-3 months*	*meet ~3-6 months*	*continuous training*	*meet ~2-3 months*	*meet ~3-6 months*

Additional Training. In addition to my experimental work, I will engage in other forms of career training:

Teaching and mentoring: Within the Dillin lab, I will continue to mentor high school, undergraduate, and graduate students, and technicians and postdocs. I will also continue working with Dr. Dillin in his molecular genetics lab in designing and carrying out a laboratory course to increase my capacity to be an efficient educator.

Increasing diversity in STEM: A large goal of mine is to increase the diversity – both of underrepresented minorities and underrepresented socioeconomic groups – in STEM. Currently, I am part of the B2B program at UC Berkeley and the CHORI/CIRM summer research programs. I plan to actively work with the heads of these programs to increase my involvement and how to incorporate these types of programs into my future institute.

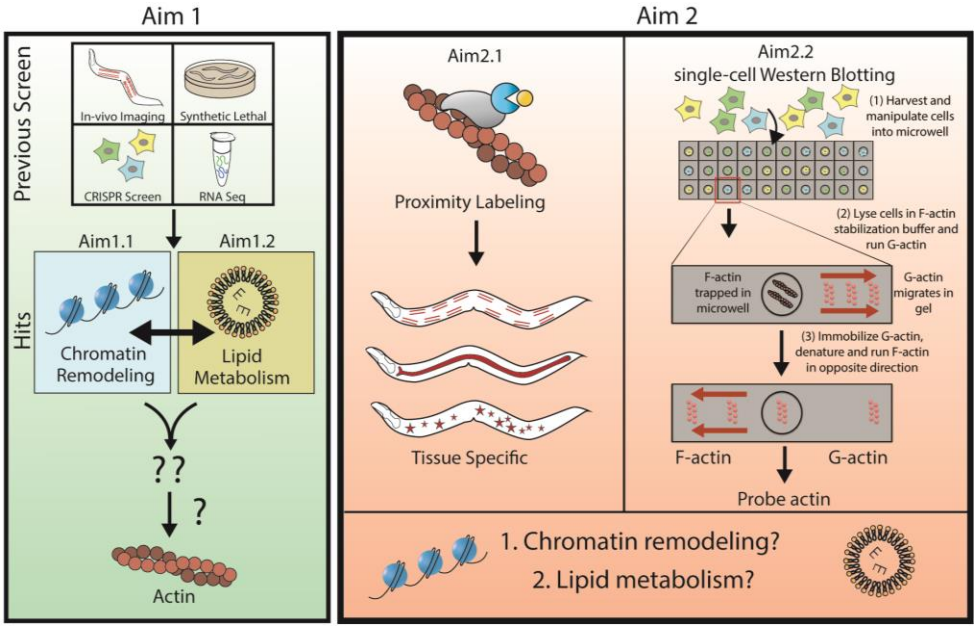
Lab management: I have a large amount of management experience both in restaurants and in retail. I had the privilege of translating these skillsets into laboratory management during my graduate work where I was serving as a secondary laboratory manager. Currently in the Dillin lab, I am expanding my expertise in lab management by working with our exceptional lab manager, Larry Joe, as he teaches me how to manage finances, personnel, and inventory of a lab. Moreover, he allows me to engage in direct negotiations with vendors.
Writing: I have had the fortune of being given the freedom to write my manuscripts independently in my graduate and postdoctoral labs. I will continue to write future manuscripts independently, with Dr. Dillin's input to refine and hone my writing skills. I have also written multiple grants both for myself, my students, and with Dr. Dillin, and write all progress reports for several grants, including my independent F32 and an R01 that I co-authored with Dr. Dillin. I will continue to expand my writing skills by co-authoring future R01 and R21-level grants.

Timelines for Career Benchmarks

Award Period	Timeline	Benchmark Goals
pre-award (Jul 2019-Apr 2020) Primary focus: -completion of post-doctoral work on ER quality control -continue preliminary work to lay the foundation for K99 proposal	June 2019	Revisions for papers on non-autonomous UPR^{ER} and TMEM's role in ER quality control submitted
	July 2019-Dec 2019	-Complete, write, and submit paper on characterizing the role of the lysosome on ER quality control (**w/ Dr. Zoncu**) -Finalize scWB techniques on quantifying F:G actin ratios (Aim 2.2 **w/ Dr. Herr**) -Complete 2nd revision/finalize papers on non-autonomous UPR^{ER} and TMEM.
	July 2019-Apr 2020	Continue experiments characterizing role of chromatin modifiers on actin health (Aim 1.1 **w/ Drs. Meyer, Eisen, & Brunet**)
	Jan 2020-Apr 2020	-Troubleshoot technique for proximity-labeling in *C. elegans* (Aim 2.1 **w/ Dr. Ting**) -Compete and submit revision of lysosome paper (**w/ Dr. Zoncu**)
K99 year 1 (Apr 2020-Mar 2021) Primary focus: -complete chromatin modifier project -Utilize scWB tools in aim questions -Begin proximity labelling project	Jun 2020-Dec 2020	Finalize work on chromatin modifiers on actin health during aging (Aim 1.1 **w/ Drs. Meyer, Eisen, & Brunet**) -Perform scWB on cells with knockouts of actin screen hits (Aim 2.2 **w/ Dr. Herr**)
	Sep 2020	Meet with advisory committee
	Dec 2020	-Submit manuscript on chromatin modifiers (Aim 1.1)
	Jan 2020-Mar 2021	-Begin proximity-labeling and send off mass spec (Aim 2.1 **w/ Drs. Ting & Phinney**) -Submit application packet for multiple faculty positions -Meet with advisory committee 2x (before & after applications)
K99 year 2 (Apr 2021-Mar 2022) Primary focus: -chromatin modifier manuscript -scWB manuscript -acquire faculty position offer -complete proximity labeling -Submit R00 transition proposal	Apr 2021-Aug 2021	Interview and negotiate for position and start-up
	Apr 2021-Aug 2021	Prepare and submit revision for paper on chromatin modifiers on actin health (Aim 1.1 **w/ Drs. Meyer, Eisen, & Brunet**)
	Aug 2021-Sep 2021	Prepare and submit R00 application (w/ **Drs. Dillin & Meyer & sponsor institution**).
	Aug 2021-Mar 2022	-Begin validation and secondary screening for hits identified in mass-spec (Aim 2.1 **w/ Drs. Ting & Phinney**) -Submit methods manuscript on scWB (Aim 2.2 **w/ Dr. Herr**)
R00 year 1 (Apr 2022-Mar 2023) Primary Focus: -Lab set-up and secure staff -Manage teaching requirements -Begin thesis projects for students	Apr 2022-Aug 2022	-Recruit personnel: hire technician, recruit undergraduate and graduate students -Write and submit methods/resource paper on proximity labeling (Aim 2.1)
	Aug 2022-Dec 2022	Fall semester lecture: molecular genetics or equivalent
	Jun 2022-Sep 2022	Prepare and submit revision for scWB paper.
	Nov 2022-Jan 2023	Prepare and submit revision for proximity labeling paper.
	Aug 2022-Mar 2023	Recruit graduate students to begin work on lipid regulators on actin health (Aim 1.2) & begin preliminary work on cross-communication between ER and cytoskeleton
R00 year 2 (Apr 2023-Mar 2024) Primary Focus: -Begin transition to new R01 work while completing R00 aims	Aug 2023-Dec 2022	Fall semester lecture: molecular genetics or equivalent
	Aug 2023-Mar 2024	Continue to recruit/train students to work on ER and cytoskeletal cross talk.
	Oct 5th 2023	Submit R01 on cross-communication between ER and cytoskeletal quality control
	Jan 2024-Mar 2024	Spring semester lab class: molecular genetics or equivalent
	Oct 2023-Mar 2024	Submit smaller grants (e.g. NSF, Glenn/AFAR, ASA, etc.)
	Feb 16th 2024	Submit R21 on riskier project following up on other hits from K99 screens
R00 year 3 (Apr 2024-Mar 2025) Primary Focus: -Secure R01 -Complete manuscripts for lipid regulation of actin and non-autonomous regulation of actin	Apr 2024-May 2024	Spring semester lab class: molecular genetics or equivalent
	July 5th 2024	Re-submit R01
	Aug 2024-Dec 2024	Fall semester lecture: molecular genetics or equivalent
	May 2024-Aug 2024	Prepare and submit manuscript on lipid regulators on actin health (Aim 1.2)
	Jan 2025-Mar 2025	Prepare and submit revision on lipid regulators on actin health (Aim 1.2)
	Mar 2025	Re-submit R21

Timeline for Research Goals

Timeline	Aim	K99 Year 1	K99 Year 2	R00 Year 1	R00 Year 2	R00 Year 3
BET-1's role in actin homeostasis and lifespan	1.1	x				
MYS-1/2's role in actin homeostasis and lifespan regulation	1.1	x				
ChIP-seq of BET-1/ATAC-seq of *mys-1/mys-2* mutants	1.1	x	x			
Characterizing HSF-1/NHR-49/DAF-16 signaling modality	1.2			x	x	
DAF-16 signaling on actin health and lifespan regulation	1.2			x	x	
Epistatic relationship of TFs on actin homeostasis	1.2				x	x
Generation and validation of turboID strains	2.1	x	x			
Tissue-specific mass-spec of actin using turboID	2.1		x	x	x	x
turboID experiments in *bet-1* and *nhr-49/daf-16* mutants	2.1		x	x	x	x
Generation and validation of scWB system	2.2	x	x			
scWB on BRD4/HNF4/FOXO mutants	2.2	x	x	x		
Adapt scWB system to *C. elegans* to study aging	2.2			x	x	x



Actin is arguably one of the most highly regulated proteins transcriptionally and translationally, popularizing it as a housekeeping gene and load control for a majority of qPCR and western blotting experiments alongside its cytoskeletal brother, tubulin. Despite this unequivocal regulation in expression, actin is one of the most dynamic proteins within the cell, consistently altering its structure, organization, and function in response to a wide range of cellular conditions. This dichotomy is explained in the plethora of actin-binding proteins involved in its fluid dynamics and regulation. The upstream network of genes responsible for regulating the expression of the many proteins responsible for cytoskeletal maintenance is poorly understood, likely owing to the complexity of cytoskeletal regulation. While our lab has identified HSF-1 function in regulation of actin during stress and aging, many actin-regulating genes are not bonafide targets of HSF-1, suggesting that other master regulators must exist, which function either independently or in parallel with HSF-1 to modulate the actin cytoskeleton. We further propose that similar to HSF-1 function, other master regulators of cytoskeletal form and function break down during the aging process, which is the key cause of cytoskeletal decline during aging. We thus propose an approach in which we dissect the genetic and molecular modulators both dependent and independent of HSF-1 in regulation of the actin cytoskeleton.

Aim 1: How does chromatin remodeling and lipid homeostasis regulate actin organization and function? We have performed multi-pronged genetic screening to identify novel regulators of the actin cytoskeleton. These studies combined *in vivo* live cell imaging of actin, synthetic lethality screening with known regulators of the actin cytoskeleton, and both transcriptome analysis and whole genome CRISPR-Cas9 screening of organisms or cells experiencing actin stress. Cross-referencing these rich datasets has revealed two critical nodes of genes: 1) modifiers of chromatin state and their downstream transcriptional regulators and 2) genes involved in lipid storage and lipid homeostasis, which we believe either work in concert or independently to maintain a chromatin state and transcriptional program that allows efficient regulation of actin. We propose to determine the impact of these factors on actin structure during the aging process in a tissue-specific manner and interrogate their impact on lifespan regulation. Moreover, we propose to study how chromatin remodeling and lipid homeostasis themselves are influenced by cytoskeletal function and dysregulation during aging.

Aim 2: Building biochemical tools to study cytoskeletal form and function during aging. Recent work from our lab has shown direct evidence that the structure and integrity of the actin cytoskeleton breaks down during advanced aging in multiple tissue. Moreover, the nature and state of the actin cytoskeleton both at young age and throughout aging is significantly different between different tissue types. Therefore, we hypothesize that the actin cytoskeleton within each tissue is regulated by a unique set of molecular interactors, which break down at different rates and complexity during aging. To test this hypothesis, we propose to employ biochemical interrogation of cytoskeletal interactors by utilizing proximity labeling methods combined with LifeAct and miniTurbo technology. This study will accomplish several goals: 1) identification of novel molecular interactors of actin that are required for cytoskeletal maintenance, 2) characterization of differences in cytoskeletal regulators across tissues, and 3) characterization of the loss of cytoskeletal interactors that result in breakdown of the cytoskeleton during aging. In addition to proximity labeling approaches, we are developing a method to biochemically interrogate cytoskeletal integrity with single cell resolution. We use single cell western blot (scWB) protocols to quantify F:G actin ratios as a proxy for cytoskeletal health. Using this system, we will determine whether the genes identified in Aim 1 also affect cytoskeletal fitness and function in human cells. Once a robust platform is built, we plan to expand this aim by adapting the method into *C. elegans* to answer additional questions: 1) Does F:G actin quantities change across age? 2) Are there tissue-specific differences in F:G actin ratios? Do they change during aging? 3) Can altering chromatin state or lipid homeostasis prevent changes in F:G actin ratios as a function of age?

Significance. The actin cytoskeleton, composed of a complex network of filaments held together by many actin-binding proteins, has historically been understood as the structural framework of the cell, with its primary function being ascribed to the sorting and transport of cellular cargo. However the proper function of actin is also required for a diverse array of cellular pathways, including autophagy, chaperone function, endocytosis, and exocytosis [1]–[5]. The breakdown of these cellular processes manifests during aging and exposure to stress, which is in part due to the breakdown of the cytoskeleton under these conditions [5]–[9]. The necessity of a properly maintained cytoskeleton in cellular homeostasis is evident in its requirement for a number of critical processes, yet the mechanisms involved in preservation of cytoskeletal form and function are not well understood.

One primary mechanism by which the cell protects its cytoskeleton during stress is through the heat shock response (HSR), mediated by the heat shock transcription factor, HSF-1. HSF-1 is activated under thermal stress and promotes protein homeostasis through upregulation of chaperones and other genes related to protein quality control [10]. Overexpression of HSF-1 is sufficient to confer thermal stress and increase lifespan in *C. elegans*, and alleviates the toxic effects associated with aging. Our lab has identified that HSF-1 also has the capacity to upregulate genes involved in maintenance of the actin cytoskeleton, including the troponin C/calmodulin homolog, *pat-10*, which is both sufficient and necessary for HSF-1-mediated thermotolerance and longevity [11]. However, many actin regulating genes are not bonafide targets of HSF-1, suggesting that other master regulators must exist, which function independently or in parallel with HSF-1 to modulate actin.

In this proposal, I will apply novel techniques to interrogate cytoskeletal quality during stress and aging to dissect the nature of cytoskeletal regulation. I employ multi-pronged genetic screening to identify critical transcriptional regulators involved in cytoskeletal regulation, and couple this with a biochemical approach to identify the molecular interactome for cytoskeletal regulation. Moreover, I propose to perform these experiments in a tissue-specific manner to understand the differences in actin regulatory proteins that allow a single protein to behave in massively different ways across tissue types. Finally, I will create a method to biochemically integrate cytoskeletal function with single-cell precision. This work will not only increase the understanding of genes and proteins required for cytoskeletal preservation, but will increase the understanding of the requirement of actin filament structure and function in the collapse of homeostasis and health during organismal aging.

Innovation. Recently, interest has increased in the mechanistic breakdown of the actin cytoskeleton throughout aging. Indeed, there exists multiple studies that correlate cytoskeletal quality with aging: premature aging models in *S. cerevisiae* show declines in cytoskeletal integrity [6]; aged muscle cells have decrease myosin function [12]; loss of regulation of filamentous (F) actin in aged T cells prevents its activation [13]; identification of cytoskeletal elements in proteome-wide analyses of age-associated changes in protein abundance in human fibroblasts [14]; and stabilization of the actin cytoskeleton results in increased lifespan in *C. elegans* [11]. *Through these indirect studies, it has been accepted as common knowledge that the actin cytoskeleton collapses as a function of age, although a direct and exhaustive characterization of actin cytoskeletal decline in multi-cellular organisms as a function of age has not been performed.* A major part of the absence of these essential studies is the lack of tools available to interrogate cytoskeletal integrity *in vivo*. I have developed a system whereby cytoskeletal integrity can be visualized specifically in multiple-tissue types in live *C. elegans*. Using these tools, *I obtained the first direct evidence that actin structure and integrity decline during late adulthood in multiple tissue types*, confirming the long-standing theory that actin faces an age-associated collapse.

My currently proposed studies are unique in that I use these novel tools to directly identify genes essential for cytoskeletal regulation. Moreover, I propose to adapt these tools even further for utility in biochemical assays to identify molecular interactors of actin in an effort to identify essential proteins for cytoskeletal regulation. While our methods are initially screen-heavy, I have already completed all the genetic screens and have even performed initial secondary and tertiary screening. To this end, I have the platforms for direct interrogation of the requirement of identified hits in regulation of actin under normal and aging physiologies. I ask direct and important questions to merge the fields of actin and aging: 1) to identify a critical chromatin and metabolic state that promotes transcriptomes characteristic of increased actin health, 2) characterization of molecular interactomes that allow for a virtually identical actin protein to function in extraordinarily different manners across tissue, and 3) to dissect the node of cellular stress response involved in preserving actin under conditions of stress and aging. Finally, I am developing a currently non-existent tool in cytoskeletal biology: biochemical interrogation of cytoskeletal quality with single-cell resolution. While this tool is proposed here to study cytoskeletal form and function during aging, it will be a pivotal tool in the field to answer currently unanswerable questions as basic as what is the cell-to-cell variability of actin form and function during cytokinesis?

Preliminary data. *I have created a system to interrogate cytoskeletal quality in vivo in C. elegans* in a tissue-specific manner by adapting the LifeAct system into *C. elegans*. LifeAct is a 17-amino acid peptide, which binds reliably to F-actin, and fusion of LifeAct to the fluorophore, mRuby, allows robust visualization of the actin

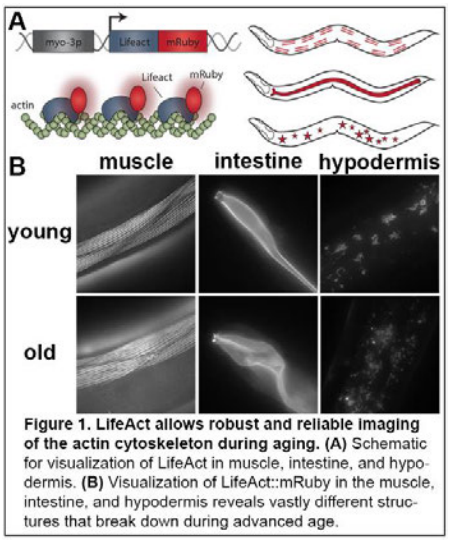


Figure 1. LifeAct allows robust and reliable imaging of the actin cytoskeleton during aging. (A) Schematic for visualization of LifeAct in muscle, intestine, and hypodermis. **(B)** Visualization of LifeAct::mRuby in the muscle, intestine, and hypodermis reveals vastly different structures that break down during advanced age.

cytoskeleton *in vivo* [15]. I created stable transgenic lines in which a single copy of LifeAct-mRuby is expressed under the control of a tissue-specific promoter in the muscle (*myo-3p*), intestine (*gyl-19p*), and the hypodermis (*col-19p*) (Fig. 1A). This peptide is sufficient to target mRuby to F-actin and exhibits none of the detrimental consequences caused by traditional visualization of actin, including actin fusions to fluorescent proteins or toxic dyes, like phalloidin [16], [17]. Using these newly synthesized strains, I performed the *first comprehensive analysis of changes to actin filaments during aging in C. elegans* [18]. The actin cytoskeleton in the muscle, intestine, and hypodermis is extremely different and all three tissue types show progressive decline of the cytoskeleton throughout aging, such that the structure and organization of actin is dramatically different between young and old worms (Fig. 1B).

The quantitative level of actin is under very tight regulation, yet the proper function of the actin cytoskeleton is dependent on the exceptional dynamic properties of the protein. This dichotomy is explained by the large number of proteins involved in regulating actin, including actin-binding proteins and factors involved in its polymerization/depolymerization. I hypothesize that this large subset of genes fall under the control of master transcriptional regulators, allowing robust activation of many actin regulatory genes by a few key proteins. HSF-1 functions as one of these factors to preserve the cytoskeleton by promoting the expression of genes involved in actin stability, including the troponin C homolog, *pat-10*, and the tropomyosin, *lev-11*. Overexpression of *hsf-1* is sufficient to protect cytoskeletal integrity at late age. Despite the identification of HSF-1 as a bonafide regulator of the actin cytoskeleton, a closer look at its downstream targets reveal that many critical components of actin maintenance are absent. In an effort to identify additional master regulators of the actin cytoskeleton, I performed a multi-pronged genetic screening approach (Fig. 2): 1) *in vivo* live-cell imaging of actin filaments to identify transcription factors required for cytoskeletal maintenance, 2) whole genome synthetic lethality screening to identify genetic interactors of *hsf-1* in *C. elegans*, 3) transcriptome analysis of *C. elegans* with sub-lethal dose of actin knockdown, and 4) whole genome CRISPR-Cas9 screening in human BJ fibroblasts to identify genes that are essential for survival under sub-lethal doses of the actin destabilizing agent, cytochalasin D. These screens have identified a number of candidate genes. Cross-referencing these rich datasets has revealed two critical nodes of genes: 1) modifiers of chromatin state and their downstream transcriptional regulators and 2) genes involved in lipid storage and global lipid homeostasis. The identification of various genes with overlapping functions across the screens provides a high level of confidence in these datasets.

Research Plan. Aim 1: How does chromatin remodeling and lipid homeostasis regulate actin organization and function? *Aim 1.1: How do chromatin modifiers alter actin form and function during aging?* To dissect the complex architecture involved in transcriptional regulation of genes involved in cytoskeletal maintenance, I propose to test the requirement of the identified chromatin modifiers in regulation of the actin cytoskeleton. *I hypothesize that a specific chromatin state is maintained to preserve a transcriptional profile, which promotes a healthy actin cytoskeleton.* Moreover, aging results in breakdown of essential chromatin modifiers, which alters chromatin state, preventing efficient transcriptional regulation of the core cytoskeletal machinery, ultimately resulting in poor actin dynamics and the physiological consequences of aging. My screens have revealed that major chromatin modifiers, such as the MYST histone acetyl transferases (HATs), are essential for maintaining a proper cytoskeletal state. I also identified, *bet-1*, a gene encoding a bromodomain protein, which exerts its transcriptional program by binding to histones acetylated by MYST HATs [19].

Aim 1.1a (K99): Can BET-1 preserve actin filaments during the aging process? I have validated BET-1 as a bonafide cytoskeletal regulator, as knockdown of *bet-1* results in increased sensitivity of actin to perturbation by heat (Fig. 3A) and a significant decrease in lifespan (Fig. 3B). Therefore, *I hypothesize that BET-1 function is essential for a healthy lifespan through its role in cytoskeletal maintenance, and that MYST HATs produce a chromatin state that allows BET-1 to exert its transcriptional program.* To further investigate the role of BET-1 on cytoskeletal health, I will quantitatively and qualitatively analyze tissue-specific patterns of actin filament structure and integrity using our LifeAct-mRuby lines. Filament structure and integrity will be qualitatively measured *in vivo* in muscle, intestine, and hypodermis of animals with knockdown or overexpression of *bet-1* throughout age at Day 1, D4, D7, D10, and D13 (*n > 50 animals per condition; >2 independent trials using 3 aging methods for >6 total replicates. Animals are aged using the following methods: 1) adults washed off plates and eggs/progeny aspirated away; 2) chemical sterilization with FUDR; 3) adults manually picked away from progeny*). Moreover, quantitative analysis can be performed in a tissue-specific manner using established methods ([18]; unpublished

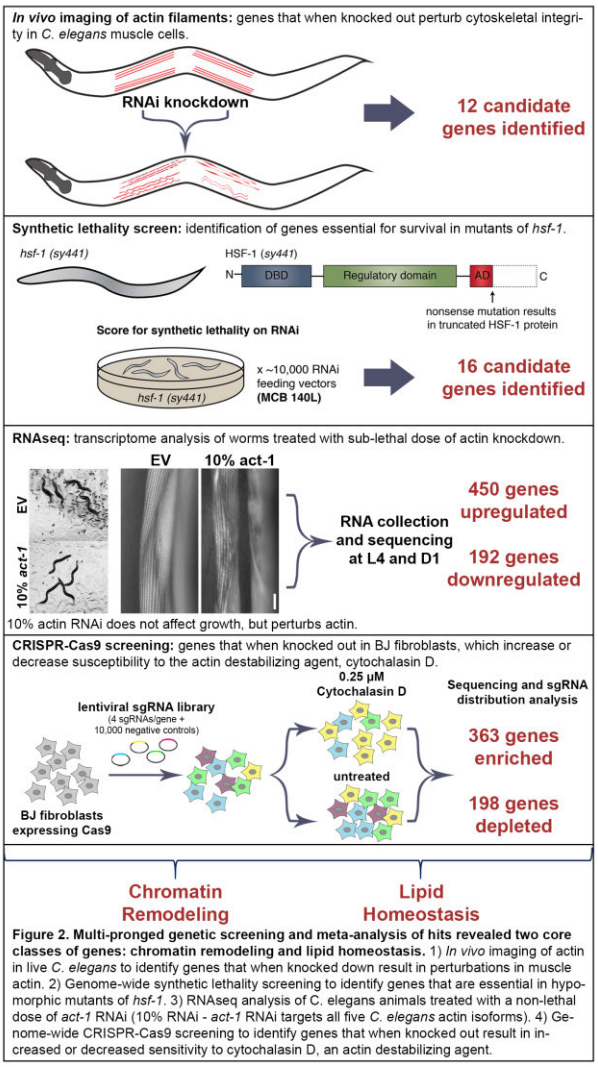


Figure 2. Multi-pronged genetic screening and meta-analysis of hits revealed two core classes of genes: chromatin remodeling and lipid homeostasis. 1) *In vivo* imaging of actin in live *C. elegans* to identify genes that when knocked down result in perturbations in muscle actin. 2) Genome-wide synthetic lethality screening to identify genes that are essential in hypomorphic mutants of *hsf-1*. 3) RNAseq analysis of C. elegans animals treated with a non-lethal dose of *act-1* RNAi (10% RNAi - *act-1* RNAi targets all five *C. elegans* actin isoforms). 4) Genome-wide CRISPR-Cas9 screening to identify genes that when knocked out result in increased or decreased sensitivity to cytochalasin D, an actin destabilizing agent.

data): 1) thickness of muscle actin filaments [20] (*n > 30 animals per condition; filaments in 1 muscle cell quantified per animal for >100 measurements*); 2) integrated fluorescent intensity of actin measured using a COPAS large particle biosorter [21]; 3) indirect measurement of intestinal F-actin using ratio of LifeAct in filaments vs. cytoplasm (*for 2 & 3: n > 500 animals per condition*); and 4) dynamics of actin measured using fluorescent recovery after photobleaching (FRAP) of LifeAct in muscle, intestinal, and hypodermal actin (*n > 30 animals per condition; 3-5 structures measured per animal for >100 measurements. Statistics run on t=1/2 measurements. For 1-4, >3 independent trials per experiment with Mann-Whitney statistical testing*). All knockdown experiments will be validated in null mutants synthesized by CRISPR-Cas9-mediated gene editing. I predict that *bet-1* knockdown will result in phenotypes associated with premature breakdown of the actin cytoskeleton, while overexpression of *bet-1* will protect the cytoskeleton in late age.

To determine whether BET-1's role in cytoskeletal integrity alters organismal physiology, I will test whether overexpression of *bet-1* can extend lifespan and survival under thermal stress, since its knockdown decreases lifespan and increases sensitivity to thermal stress (*lifespans & thermotolerance on >120 adult animals per condition; >3 independent trials; log-rank testing for significance*). Next, I will determine whether BET-1-mediated lifespan extension is dependent on proper actin function to eliminate the possibility that BET-1 alters physiology through an alternative, actin-independent function. I will treat *bet-1* overexpressing strains with RNAi targeting actin and known actin-regulating genes, such as tropmyosins, the ARP2/3 complex, and formins, to determine whether loss of broad and specific actin regulatory genes is sufficient to attenuate the lifespan extension found in *bet-1* overexpressing animals. *CAVEAT 1*: if *bet-1* overexpression does not extend lifespan, these proposed experiments can be done in *bet-1* knockdown or lof animals to determine whether their effects on lifespan or thermotolernace is additive (suggesting independent mechanisms) or not (suggesting similar mechanisms). *CAVEAT 2*: it is possible that massive overexpression of *bet-1* may be toxic, and thus titrating *bet-1* levels may be necessary for lifespan extension. Therefore, the following experiments will be performed as necessary: 1) single-copy overexpression of *bet-1* using mosSCI for lower expression to rule out the possibility that high levels of *bet-1* is toxic, 2) tissue-specific overexpression of *bet-1* to test the possibility that *bet-1* overexpression is beneficial only in some tissue, but toxic in others, 3) overexpression of specific isoforms of *bet-1* to test the hypothesis that each isoform has a unique role in physiology.

Aim 1.1b (K99): Is BET-1's role in cytoskeletal maintenance dependent on MYST HAT-mediated chromatin remodeling? Several MYST HATs were identified as genes essential for survival under actin destabilizing conditions in our screens. MYST HATs target H4 [22], [23] and their histone acetylation function have been ascribed to transcriptional activation in drosophila [24] and yeast [25]. In *C. elegans*, there are four primary MYST HATs, *mys-1 through 4*, and two putative MYST HATs, *C34B7.1* and *C34B7.2*. *mys-1*/*mys-2* are upstream of BET-1 and acetylate H4 at K5, K8, K12, and K16 [19]. These acetylation states allow BET-1 binding to H4 and subsequent transcriptional activation to promote cell-fate decisions. To determine whether BET-1 function in cytoskeletal maintenance follows the same molecular mechanism as cell-fate decisions, I propose to interrogate cytoskeletal quality in animals with *mys-1* and *mys-2* knockdown using similar strategies proposed in Aim 1.1a. If BET-1 impacts actin health through its canonical role as a transcription factor, knockdown of either *mys-1* or *mys-2*, which prevents localization of BET-1 to DNA, should mimic defects in cytoskeletal health and lifespan found in *bet-1* knockdown. Moreover, knockdown of *bet-1* should not further abrogate cytoskeletal health or lifespan in *mys-1* or *mys-2* knockdown animals. Finally, if I see that *bet-1* overexpression improves cytoskeletal quality and lifespan in Aim 1.1a, I will determine whether *mys-1* or *mys-2* knockdown can suppress the beneficial effects of *bet-1* overexpression. As an extension to this aim, I will also test the role of the two putative MYST

HATs, *C34B7.1* and *C34B7.2* in regulation of actin and lifespan as these genes also came up in our screens. *CAVEAT:* It is completely possible that BET-1's role in cytoskeletal maintenance is completely independent of MYST HAT-mediated chromatin remodeling for two reasons: 1) BET-1 transcriptional regulation of cytoskeletal genes are independent of H4 acetylation as its binding is distinct from this mechanism. This hypothesis will be tested in the proposed experiments above, and further testing of BET-1's function as a transcription factor are proposed below in Aim 1.1c-d (CHIP-seq, ATAC-seq, and RNAseq experiments). 2) BET-1's role in cytoskeletal maintenance are independent of its role as a transcription factor. If this holds true, overexpression of *bet-1* lacking a nuclear localization signal should be sufficient to protect cytoskeletal integrity and extend lifespan, and Aim 1.1a/c/d will be performed using *bet-1DBD*△ overexpressing animals.

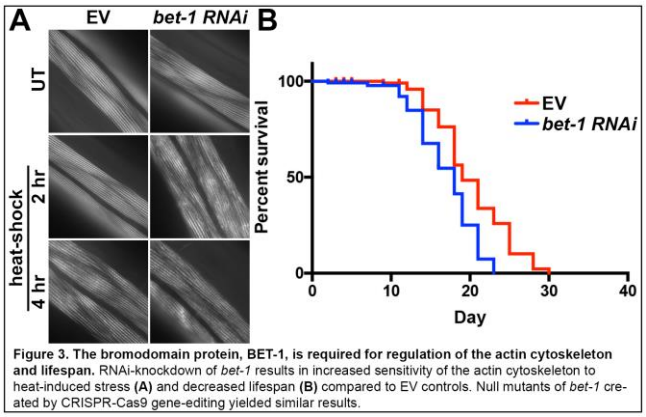


Figure 3. The bromodomain protein, BET-1, is required for regulation of the actin cytoskeleton and lifespan. RNAi-knockdown of *bet-1* results in increased sensitivity of the actin cytoskeleton to heat-induced stress **(A)** and decreased lifespan **(B)** compared to EV controls. Null mutants of *bet-1* created by CRISPR-Cas9 gene-editing yielded similar results.

Aim 1.1c (K99): Does functional activity of BET-1 decline during aging? The functional activity of MYS-1 and MYS-2 is essential in maintaining BET-1 localization to the nucleus where it exerts its function as a transcription factor [19]. *It is possible that defects in actin during aging are due, at least in part, to functional decline of BET-1 during the aging process*. To test this hypothesis, I will determine whether nuclear localization of BET-1 declines during aging. A previous study has shown that BET-1::GFP fusion proteins maintain full function and its nuclear localization is a reliable marker for its transcriptional activity [19]. I will test BET-1::GFP localization in animals at D1, D4, D7, D10, and D13 (*n > 50 animals per condition; >2 trials per aging method for >6 total replicates. Quantification measured as % integrated fluorescence of nucleus:cytoplasm. Mann-Whitney statistical testing*). To directly test BET-1 transcriptional activity, I propose to perform ChIP-seq (chromatin immunoprecipitation sequencing) of BET-1 (all ChIP-seq will be performed with the guidance of Drs. Meyer and Eisein; *5 biological replicates of >500 animals for all sequencing analysis – ChIP-seq, RNAseq, and ATAC-seq as per Vincent J. Coates sequencing core suggestions, where all sequencing analysis will occur. Data analysis will be performed with the help of the Meyer and Eisen lab. Reads will be mapped using bowtie2, and SAMtools, deepTools, and macs2 for calling peaks*). It is likely that during the aging process, BET-1 transcriptional activity is perturbed via decreased BET-1 recruitment to loci coding for actin-regulatory proteins, which results in the breakdown of the cytoskeleton with age. If this holds true, BET-1 function may decline due to the functional breakdown of MYS-1 and MYS-2 in maintaining a core chromatin state for BET-1 binding. Thus, I will test whether any measured breakdown of BET-1 function can be rescued by overexpression of *mys-1* or *mys-2*, and whether this can protect the actin cytoskeleton during aging to promote organismal lifespan. *CAVEAT*: It is possible that overexpression of *mys-1/mys-2* may be detrimental due to off-target effects of systemic changes in chromatin acetylation state. Therefore, general chromatin state can be measured throughout aging using ATAC-seq (assay for transposase-accessible chromatin using sequencing; will be performed with the guidance of Drs. Meyer and Eisen; *bowtie to map reads, SAMtools, bedtools, and MACS for calling peaks*). I will perform ATAC-seq on animals during the aging process (D1, D4, D7, D10, D13) to determine what changes occur in chromatin state and chromosome availability during the aging process. Then I will compare this to knockdown of *mys-1* and *mys-2* to see if knockdown of these critical acetylation factors can phenocopy the deterioration of chromatin state during aging. Moreover, Studying global chromatin accessibility in animals with knockdown of *mys-1* and *mys-2* will give us an understanding of chromatin state under conditions when BET-1 is functionally inactive. *CAVEAT 2*: If BET-1::GFP and ChIP-seq experiments do not yield measurable changes during age, I will perform RNAseq in *bet-1* knockdown and overexpression animals to determine whether major transcriptional changes exist in cytoskeletal genes (*Reads will be aligned and quantified using salmon with WBcel235 as worm reference genome. Fold changes determined using R-package DESeq2*). Subsequent promoter mining of cytoskeletal targets may reveal putative transcription factor binding motifs of BET-1 for cytoskeletal genes.

Aim 1.2 (R00): How does lipid metabolism alter cytoskeletal integrity? Largely, aberrant metabolic state affects cytoskeletal regulation. For example, improper nutrient availability can perturb cytoskeletal dynamics through mTORC1 signaling [26]. This is unsurprising due to the high energy demands of maintaining proper cytoskeletal form and function. In our screens, I have found that several regulators of general metabolism – with an enrichment of genes involved in lipid metabolism – were essential for proper function of the actin cytoskeleton. Interestingly, I have found that RNAi knockdown of the *daf-16*/FOXO transcription factor, involved in insulin signaling, perturbs actin filament organization in the muscle. Our previous work has identified that *daf-16* is

critical for the beneficial effects of non-autonomous HSF-1 signaling [27]. I have also identified *nhr-49*/HNF4, previously characterized for its role in β-oxidation and fatty-acid desaturation [28] (Fig. 4A).

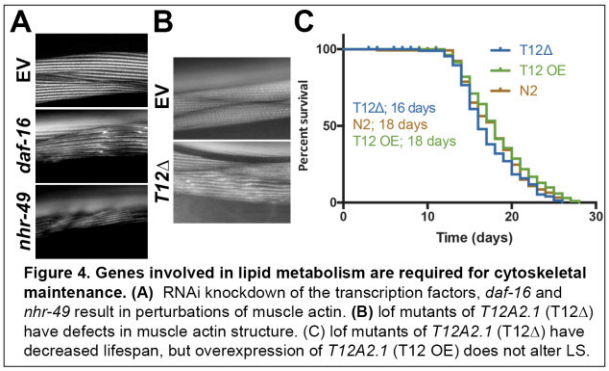


Figure 4. Genes involved in lipid metabolism are required for cytoskeletal maintenance. (A) RNAi knockdown of the transcription factors, *daf-16* and *nhr-49* result in perturbations of muscle actin. **(B)** lof mutants of *T12A2.1* (T12Δ) have defects in muscle actin structure. (C) lof mutants of *T12A2.1* (T12Δ) have decreased lifespan, but overexpression of *T12A2.1* (T12 OE) does not alter LS.

Aim 1.2a (R00): Are master regulators of lipid homeostasis required for cytoskeletal health during aging? Our previous work identified a link between *hsf-1* and *daf-16*; however, this is the first time that a direct relationship between actin filaments and *daf-16* has been identified. Similarly, while energy deprivation and ATP availability have been correlated with actin function, no direct relationship between *nhr-49*/HNF4 has been described. Importantly, overexpression of *daf-16* and *nhr-49* extends lifespan in *C. elegans* [28], [29]. Therefore, *I hypothesize that DAF-16/NHR-49 promote lifespan through its role in regulation of actin.* To test this hypothesis, I will measure actin form and function during the aging process in animals with *daf-16* or *nhr-49* overexpression as described in Aim 1.1a. Moreover, I will determine whether *daf-16* and *nhr-49*-mediated lifespan extension is dependent on functional actin by determining whether RNAi knockdown of actin regulators suppress the lifespan effect of *daf-16* or *nhr-49* overexpression. In parallel, I will determine whether mutants of *daf-2*, the IGF-1/IIS receptor, also protect cytoskeletal health late into adulthood, as these mutants exhibit one of the highest recorded lifespans by activating DAF-16 [30].

CAVEAT/Alternative hypothesis: In our studies, I found that loss of genes involved in global lipid homeostasis, such as *nhr-49*, *daf-16*, and *T12A2.1*, result in defects in actin structural integrity (Fig. 5A-B). Moreover, previous reports have found that mutants of *nhr-49* have elevated fat content and decreased lifespan [31]; DAF-16 promotes longevity through transcriptional regulation of genes involved in both lipid synthesis and breakdown [32]; and mutants of T12A2.1 have decreased lipid levels [33] and exhibit decreased lifespan (Fig. 5C). Through these, we hypothesized that lipid dysregulation can affect cytoskeletal form and function. However, I also propose to test the reverse hypothesis that a properly functional actin cytoskeleton is essential in regulation of lipid homeostasis, and that cytoskeletal breakdown can be a factor in lipid dysregulation during aging.

A properly functional cytoskeleton is essential for many processes, such as mediating organelle dynamics of the mitochondria [6] and ER-mitochondria contacts [34], [35]. Moreover, F-actin/G-actin levels can alter signaling of the major transcriptional regulator, serum response factor (SRF), which can impact many different pathways including cell cycle, apoptosis, cell growth, and mitochondrial dynamics [36], [37]. Interestingly, I find that perturbations to the actin cytoskeleton via RNAi knockdown of actin itself or a tropomyosin, lev-11, results in increased lipid droplet numbers in the muscle, which is a similar phenotype that I find in aged animals (data not shown). I propose to measure organismal lipid levels in animals with defective actin cytoskeleton by using BODIPY and Nile Red staining using fluorescent microscopy and flow cytometry with a COPAS biosorter (*n > 500 animals per condition; >3 independent trials; Mann-Whitney testing for statistics*). Next, to determine whether protecting the cytoskeleton during aging can prevent an age-associated decline in lipid homeostasis, I propose to measure lipid droplet morphology and whole animal lipid levels in animals with a protected cytoskeleton during aging (using hsf-1 overexpressing animals and any other overexpression line that I find in this study to protect the actin cytoskeleton; *for lipid droplet morphology, >50 animals per sample; >10 lipid droplets analyzed per animal for > 500 measurements; analyses made on size, density, and quantity of lipid droplets; Mann-Whitney testing for statistics*). Finally, I will determine whether proper cytoskeletal function is required for NHR-49 and DAF-16-mediated effects on lipid homeostasis. In Aim 1.2a, I propose to test whether knockdown of essential cytoskeletal components could suppress the lifespan extension found in these animals. Here, I extend this study to determine whether knockdown of similar cytoskeletal components can perturb the lipid phenotypes found in these animals, including decreased fat content (measured using BODIPY and Nile Red staining) and increased β-oxidation and fatty acid desaturation (*using qPCR against canonical targets upregulated by NHR-49* [28]; *all qPCR experiments are performed with 4 technical replicates of each 3 biological replicate collected from > 1,000 animals. qPCR measurements are made against a standard curve and normalized to major C. elegans house-keeping genes, pmp-3, sap-49, and Y45F10D.4; a two-group t-test will be used for statistical analysis*).

Aim 1.2b (R00): Does lipid homeostasis exert its effects on actin and aging through chromatin regulation? In my genetic screens, I found enrichment in two molecular pathways: chromatin remodeling and lipid homeostasis. There exist a growing body of evidence that lipids can indirectly impact chromatin states (reviewed in [38]), potentially linking these seemingly disparate classes of genes. For example, fatty acid oxidation acts as a source of acetyl-CoA, which can be utilized by HATs in histone acetylation [39]. Thus, *there is a possible linear mechanism by which NHR-49/DAF-16 alter lipid metabolism to promote chromatin remodeling by MYS-1/MYS-*

2, allowing BET-1 to create a healthy actin transcriptome. To test this hypothesis, I propose both a directed and broad interrogation of chromatin state in animals with altered lipid homeostasis. First, I will test whether knockdown or overexpression of *nhr-49* alters H4 acetylation, the primary target of MYS-1/MYS-2. It is possible that NHR-49-mediated beta-oxidation is a critical source of acetyl-CoA for acetylation of H4 by these HATs, such that knockdown of *nhr-49* will result in decreased H4 acetylation, and overexpression of *nhr-49* will result in increased H4 acetylation. (*H4 acetylation status will be tested by H4 immunoprecipitation followed by mass-spec* [23]; *5 biological replicates of >100,000 animals will be collected and analyzed with Dr. Phinney)*. I will extend these studies to animals with knockdown or overexpression of *daf-16* to determine whether DAF-16 can affect histone acetylation. In addition, I plan to perform BET-1 localization experiments and ChIP-seq of BET-1 as described in Aim 1.1c to determine whether NHR-49/DAF-16 modulates BET-1's transcriptional activity. *CAVEAT*: If changes in *nhr-49/daf-16* expression do not alter H4 acetylation or BET-1's function, it is still possible that *nhr-49/daf-16* alters larger, global chromatin states, which may affect cytoskeletal health independent of MYS-1/MYS-2/BET-1. Therefore, I propose to interrogate global changes in chromatin state by performing ATAC-seq in animals with knockdown or overexpression of *nhr-49*/*daf-16.* These studies may reveal a potential mechanism merging the functional roles of lipid homeostasis and chromatin remodeling on cytoskeletal health.

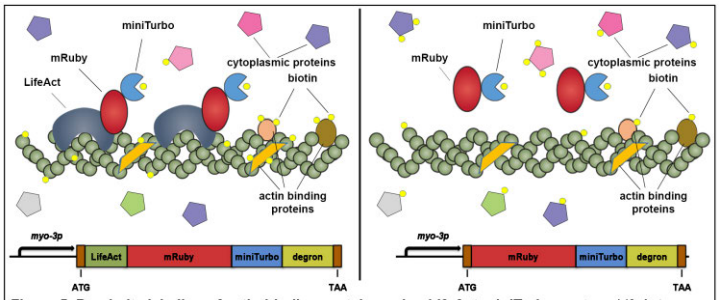


Figure 5. Proximity labeling of actin-binding proteins using LifeAct-miniTurbo system. LifeAct::mRuby::miniTurbo is expressed using tissue-specific promoters, such as myo-3p visualized here for the muscle. The construct will be degraded when animals are supplemented with auxin, and animals will be released from auxin to allow temporal resolution of proximity labeling. Cytoplasmic noise will be erased by subtracting proteins enriched in animals expressing mRuby::miniTurbo without LifeAct.

Aim 2: Building biochemical tools to study cytoskeletal form and function during aging. The nature and state of the actin cytoskeleton, both at young age and throughout aging, is significantly different between tissue types, despite the actin protein being virtually identical. Therefore, I hypothesize that the actin cytoskeleton within each tissue is regulated by a unique set of molecular interactors, which break down at different rates and complexity during aging. While I have developed tools to qualitatively answer these questions through imaging, biochemical tools to assay cytoskeletal health and the molecular players involved, are completely lacking. Here, we propose to develop two novel tools to interrogate the actin cytoskeleton.

Aim 2.1: *Development of proximity-dependent protein labeling of actin (K99).* Proximity labeling using BirA and other similar biotin-tagging enzymes have been used to robustly map proteomes of specific cells, organelles, and subdomains without a need for purification or fractionation [40], [41]. The Ting lab has performed directed evolution to produce miniTurbo, a mutant of biotin ligase with greater efficiency in proximity labeling [42]. miniTurbo allows robust biotinylation of proteins in close proximity to the enzyme, with less background and higher specificity than similar constructs. I propose to employ a *biochemical interrogation of cytoskeletal interactors by utilizing miniTurbo in combination with LifeAct technology* (with the guidance of Dr. Alice Ting). I have already established LifeAct in *C. elegans* as a reliable method to visualize F-actin in a tissue-specific manner by fusion to a fluorescent molecule. I propose to adapt this technique by fusing LifeAct to miniTurbo (Fig. 6). I employ tissue-specific promoters as I did for our original LifeAct constructs for spatial control. For temporal control, I have attached a degron tag to the construct, which allows degradation of the protein when animals are supplemented with auxin [43]. Animals will be removed from auxin 24 hours prior to collection of protein for robust, temporally controlled, biotinylation of proteins. Currently, I have created transgenic lines carrying a single-copy integration of LifeAct::mRuby::miniTurbo::degron in the muscle, intestine, and hypodermis, and see that this construct reliably localizes to the actin cytoskeleton without perturbations in cytoskeletal structure. Currently, I am creating identical lines with LifeAct removed. This cytoplasmic mRuby::miniTurbo::degron system will allow us to identify cytoplasmic proteins, which can be removed from our dataset.

After confirming spatial and temporal control and efficacy of our miniTurbo constructs, I will purify biotinylated proteins to identify actin interacting partners using tandem mass tag (TMT) labeling coupled to quantitative MS/MS analyses. TMT labeling allows multiplexing of up to 10 samples in a single MS/MS run [44]–[46]. I will first test the ability to pulldown proteins that are in close proximity to LifeAct by probing pulldowns with anti-actin antibodies. My LifeAct pulldowns should have massive enrichment for actin compared to the cytoplasmic pulldowns. Next, I will subject the samples to TMT labeling and send them to UC Davis for MS/MS analysis. The proteomic analysis workflow will be performed with Brett Phinney who will perform the mass spectrometry and analysis using X! Tandem, Scaffold Q, and SafeQuant softwares (*As agreed with Dr. Phinney, we will run analysis on 6 independent replicates of > 100,000 worms per sample*). I will also work closely with the Ting lab to learn how to analyze mass spectrometry data independently as more data is collected.

This tool will be used to determine the molecular interactomes that result in tissue-specific form and function of actin. I hypothesize that cross-comparison of molecular interactors of actin between different tissue types will reveal the critical interactome of actin required to exhibit tissue-specific form and function of immensely variable dynamics of actin. Indeed, I find that utilization of actin destabilizing drugs that target different forms of actin do not affect actin integrity equally in all tissues. For example, I find that the muscle actin is highly insensitive to CK-666, an arp2/3 inhibitor, and is only affected by long-term treatment with cytochalasin D, a drug that binds F-actin preventing polymerization of monomeric actin onto F-actin ends, and latrunculin A, a drug that sequesters g-actin to prevent further polymerization (data not shown). These data suggest that *actin in the muscle is less branched and more linear in nature, and is highly stable*. In contrast, hypodermal actin is highly sensitive to every actin destabilizing drug tested (CK-666, cytochalasin D, latrunculin A, and SMIFH2, a formin inhibitor), while intestinal actin is highly insensitive to these drugs. I also confirmed these results using genetic perturbations of cytoskeletal regulators by RNAi. This suggests that hypodermal actin is very dynamic and contains both branched and linear actin filaments, while intestinal actin is a unique and hyper-stable form of actin.

To test these hypotheses, I will use the method here to identify molecular interactors of the actin cytoskeleton in a tissue-specific manner and across various time points in aging. I will start with the muscle, intestine, and hypodermis, which I have already verified have robust labeling of actin using LifeAct. I will perform pulldowns and mass-spec at young and old adult animals at various stages where I have already characterized a healthy and dysfunctional cytoskeletal network (see Fig. 1B for an example, and [18] for comprehensive analysis). For muscle and intestine, I will collect samples at D1 and D10, and for the hypodermis I will collect samples at D3 and D7. As a positive control, I will collect samples of young-age animals treated with a condition, which I have already validated perturbs cytoskeletal integrity to a similar extend to aging (tropomyosin knockdown for muscle, arp-2/3 inhibition for hypodermis, and actin knockdown for intestine). While I expect these lists to be expansive, our lab has expertise in massive, large-scale screens to interrogate cytoskeletal quality as described in Aim 1. I can very quickly and efficiently perform RNAi screens to identify genes, that when knocked down, perturb cytoskeletal integrity in the muscle, intestine, and hypodermis, and perform synthetic lethality screening with mutants of *hsf-1* or animals with exposure to actin-destabilizing chemicals, heat, or actin knockdown (using similar methods as Aim 1). For those genes that show changes to cytoskeletal integrity in at least one tissue, I will measure their impact on longevity. *CAVEAT*: During the aging process, tissue-specific promoters become leaky and it may be difficult to ascertain the breakdown of the cytoskeleton in a tissue-specific manner at advanced age. Therefore, as an alternative aim and expansion to this study, we will perform proximity labeling in animals harboring a knockdown or overexpression of genes identified in Aim 1. We will determine the impact of altering chromatin state (*mys-1/mys-2/bet-1* knockdown and overexpression) and lipid metabolism (*nhr-49/daf-16*) on the actin interactome and determine whether these differences contribute to aging.

Aim 2.2: Development of single cell western blotting (scWB) protocols to quantitatively interrogate cytoskeletal function. Actin has critical functions in cell motility, proliferation, and differentiation when it polymerizes from globular (G) to filamentous (F) actin. Therefore, F:G actin ratios can be used as a measurement of the functional output of the actin cytoskeleton. However, tools to quantify F- and G-actin are limited. Staining of F- and G-actin yields fixation artifacts and is semi-quantitative due to competitive binding between stains and actin binding proteins. Bulk centrifugation assays where F-actin is pelleted away from G-actin requires a large amount of sample, requires heavy handling and manipulations of samples that can introduce artifacts, and is a bulk summary of hundreds of thousands of cells and fails to measure the inherent cell-to-cell variability within a population. In collaboration with the Herr lab, I propose to create a new tool using electrophoretic cytometry as a first-in-kind microscale assay for electrophoretic separations of F and G actin from single cells (Fig. 7).

scWBs are capable of measuring cell-to-cell variation in protein functions using a microfuidically instrumented microscope slide [47]. Cells are settled into microwells, lysed directly in the well, and then subjected to any set of blotting on a light-activated polymer matrix. Here, we are adapting a serial lysis technique using different detergents. First, cells will be lysed in an F-actin stabilization buffer, which will lyse cells while maintaining F-actin in its polymerized state. An electrical current will be run in one direction, allowing G-actin to enter the polyacrylamide matrix, but F-actin is size-excluded. G-actin is immobilized in the gel by UV, then a denaturation buffer will be applied to depolymerize F-actin, and a current will be run in the opposite direction to allow all actin species in an F-actin state to resolve on the left side of the gel. Then standard immunoprobing can be performed to quantify F- and G-actin within hundreds of cells in a single slide. To validate the assay, we are performing the assay with MDM-MBA-231 breast cancer carcinoma cells and the karyotypically normal BJ fiobroblast cell lines. To date, we have accomplished separation of F:G actin species in both cell types and can robustly and reproducibly quantify F and G actin using standard actin antibodies. We have collected data on over 22 replicates for these cell types with sample sizes ranging from 43-234 cells per replicate, and find no

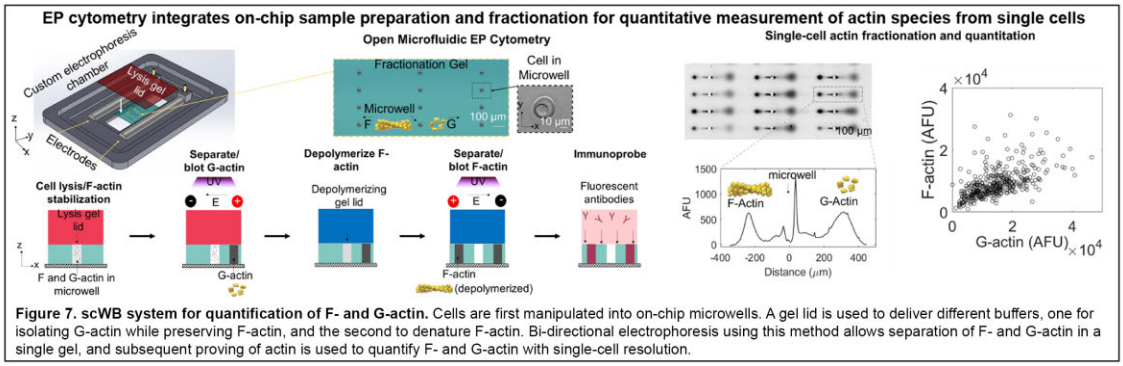


Figure 7. scWB system for quantification of F- and G-actin. Cells are first manipulated into on-chip microwells. A gel lid is used to deliver different buffers, one for isolating G-actin while preserving F-actin, and the second to denature F-actin. Bi-directional electrophoresis using this method allows separation of F- and G-actin in a single gel, and subsequent proving of actin is used to quantify F- and G-actin with single-cell resolution.

significant difference across most replicates using Kruskal-Wallis testing (data not shown). To further validate our results, we propose to test cells treated with actin stabilizing drugs, such as jasplakinolide, to increase F:G actin ratios, and actin-destabilizing drugs, such as latrunculinA, to decrease F:G actin ratios. We currently have shRNA and CRISPR-knockout lines of BRD4 (*bet-1* homologue), KAT5 (*mys-1* homologue), HNF4 (*nhr-49* homologue), and HSF1. We plan to test the conservation of these genes in regulating cytoskeletal function, as measured by F:G actin ratios, in human cells (*n > 200 cells per condition. We will run samples to ensure >5 replicates show nonsignificant differences using Kruskal-Wallis testing for each mutant tested to ensure that run-to-run variability/noise is insignificant compared to strain-to-strain differences*). If our hypotheses described in Aim 1 hold true, then knockdown/knockout of BRD4, KAT5, HNF4, and HSF1 should result in decreased F:G actin ratios. *CAVEAT 1:* Because the current assay is developed on adherent cells, it is possible that the trypsinization process used to move cells into microwells causes significant changes to cytoskeletal structures, which may introduce artifacts and confound measurements. Therefore, we are currently optimizing two additional methods: 1) using suspension Jurkat cell-lines determine whether trypsinization produces irreproducible artifacts, and 2) on-chip culturing and lysis of cells to avoid harmful preparation steps. Here, we use ECM-coated polyacrylamide gels on top of which a polyacrylamide gel microwell array can be fabricated. Cells can thus be cultured directly within the microwell, and we find that BJ fibroblasts adhere and grow well in these chambers.

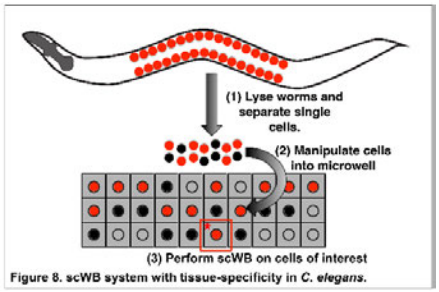


Figure 8. scWB system with tissue-specificity in *C. elegans*.

CAVEAT 2: It is possible that the molecular mechanism governing chromatin remodeling through BRD4/KAT5, or lipid homeostasis through HNF4, do not alter actin cytoskeletal state in human cells, due to lack of conservation of these molecular pathways, or different and/or redundant functional homologues of these genes in human cells. Therefore, as an extension to this aim, we propose to adapt the scWB system into *C. elegans* to further test our hypotheses proposed in Aim 1. For *C. elegans*, the major challenge is the thick cuticle of the worm, which would make in-well lysis impossible. Therefore, instead of using whole worms, we propose to use single-cells isolated using existing protocols [48]. This provides two major advantages: 1) microwell arrays developed for human cells can be used, saving time in fabrication and trouble-shooting of new microwells, and 2) tissue-specific studies can be performed. Specific cells are separated by lysing worms in gentle detergents, then sorting cells of interest with FACS (LifeAct::mRuby lines will be used). The method of separating cells is robust, and has been characterized heavily to be used for tissue-specific RNAseq measurements, and thus we believe it would be more than sufficient for our studies here [48]. Separated cells will be settled into microwell arrays as described above, and subject to scWB analysis (Fig. 8). We will first verify that this assay can defect the shift away from F-actin and towards a predominance of G-actin with age. We will extract muscle, intestinal, neuronal, and hypodermal cells from *C. elegans* populations at intervals during adulthood (D1, D4, D7, D10) and subject them to scWB using >100 cells and the same statistical rigor described above. We predict that all cell types should display a decline in F:G actin ratios as a function of age, although potentially at variable rates. Once this is confirmed, we will perform similar analyses in animals with *bet-1/mys-1/mys-2 or nhr-49/daf-16/T12A2.1* knockdown or overexpression to test the role of chromatin remodeling and lipid metabolism on actin function.

Summary. Regulation of the actin cytoskeleton is a complex and multi-faceted process. This proposal uses an in depth approach to dissect the process of cytoskeletal regulation during aging. I will determine the role of chromatin remodeling and lipid homeostasis on cytoskeletal regulation and map the molecular interactome of the cytoskeleton in a tissue-specific manner. Finally, the biochemical tools synthesized here will provide an incredible means to answer diverse questions both in aging and in cytoskeletal biology.

Rigor of experimental design is incorporated in each aim (see italicized). All qualitative measurements, including lifespans and microscopy, will be double-blinded. All animals and samples will be assigned at random. All replicates and sample sizes will be provided for all analyses to provide transparency. All questions will be interrogated using multiple independent approaches to ensure against biases of a single method.

Responsible Conduct of Research

1. **Format**: I have participated in a formal training course at Berkeley known as the RCR training course that has five face-to-face class meetings going over all the topics required for the RCR (this course meets all the requirements of the NIH RCR and information is available at http://extension.berkeley.edu/rcrtraining/). This class was offered through the Helen Wills Neuroscience Institute and taught by Victoria Sharma. I plan to retake the course in 2019-2020 should I receive the K99/R00 award. Informal training will also take place as face-to-face discussion and training between myself and other fellows, scholars, participants, and the principal investigator in the lab. In addition, I will utilize the online training curriculum at the Collaborative Institutional Training Initiative (CITI) website to solidify internalization of the material learned in the RCR course.
2. **Subject Matter**: The following topics will all be covered in the training:
    a. conflict of interest
    b. ethical use of animals
    c. history of humans and research
    d. data management and access to research tools
    e. responsible conduct of research
    f. mentorship
    g. authorship and publication
    h. stem cell research
3. **Faculty participation**: The training faculty (principal investigator Dr. Andrew Dillin) will participate directly in the training Informal instruction will occur throughout the duration of the fellowship as interaction in the laboratory, as well as outside the lab during weekly meetings.
4. **Duration of instruction:** The RCR course at Berkeley will exist as five face-to-face class meetings of minimally two hours in duration, and serve as the primary training of the RCR subject matters as listed above. There will also be constant informal training as interactions with other fellows and faculty in the lab throughout the fellowship, and as online courses taken minimally twice monthly to solidify learning of matters covered in the course.
5. **Frequency of Instruction:** During my graduate work at Columbia University, I attended a Responsible Conduct in Research course during my first year as a graduate student (2012). The course was organized and directed by Dr. Richard Kessin. It was a 10-week course with weekly 1-hour meetings to discuss topics of relevance in research ethics. The classes were organized as small group meetings where students and faculty engaged in conversations incorporating many topics: ethical use of animals, conflict of interest, history of humans and research, data management and access to research tools, responsible conduct of research, mentorship, authorship and publications, and stem cell research. As a postdoctoral scholar at University of California, Berkeley, I have attended a similar course in 2017 with Victoria Sharma, and plan to retake the course in 2019-2020 should I receive the K99/R00 award.

(I) Sponsor Information

a. Research Training Plan

Ryo has received exceptional training as a graduate student in Dr. Liza Pon's lab. There, he studied the functional role of the actin cytoskeleton in mediating quality control of mitochondria, and how this impacted lifespan. Through these studies, Ryo has become a great cell biologist with excellent microscopy techniques. He brought these skillsets into my lab, and transitioned his studies into understanding the complex genetic and molecular network in regulation of the actin cytoskeleton. I was impressed in how quickly and efficiently he learned about the biology of proteostasis and stress response, and how he evolved and combined this work into his interest on the actin cytoskeleton. In the first three years of his postdoc, Ryo has mastered genetic tools in both *C. elegans* and in human tissue culture, and he will spend the K99 portion of the grant focusing on increasing his understanding of large data science, including RNA-seq, ATAC-seq and ChIP-seq analyses. For these efforts, he will work with Dr. Raz Bar-Ziv, Larry Joe, and Dr. Erica Moehle, who are all members of my lab that have expertise in large data analysis using R, matlab, and python. He will also work closely with Dr. Barbara Meyer, an expert in chromatin biology, who has agreed to be a co-sponsor (see below).

Ryo has also collected a panel of experts for his committee. Dr. Michael Eisen is a chromatin and gene expression expert, and the members of his lab will aid him along with the Meyer lab in developing both ATAC-seq and ChIP-seq experiments in *C. elegans*. These methods already exist and are commonly used in the worm model, and the Eisen lab regularly uses these methods in drosophila. Ryo will work closely with members of the Eisen lab, including staff scientist, Xiao-Yong Li, and postdoc, Colleen Hannon, who have expertise in analyses of enhancers and transcriptional regulation. With close collaboration with the Eisen lab, Ryo will increase his knowledge in these important techniques, and learn how to handle large datasets using computational methods. Ryo will also work closely with Dr. Amy Herr, whose lab has made tremendous advances in single-cell immunoblot tools. Ryo has been collaborating with the Herr lab for about two years on a very exciting project in adapting a method to biochemically interrogate cytoskeleton fitness with single-cell resolution. He will continue this incredible collaborative effort as stated in his proposal, where he will gain an enormous amount of mentorship, training, and support directly from Dr. Herr and the rest of her lab. Currently, he is working directly with a graduate student, Louise Hansen, and postdoc, Dr. Julea Vlassakis, who provide tremendous support in increasing his expertise in biochemistry and bioengineering. For proteomic analysis, Ryo will work with the members of the laboratory of Dr. Alice Ting. He will work directly with Tess Branon, who has pioneered the work on miniTurbo, the method he plans to use for his proximity labeling. Moreover, Alice has agreed to allow Ryo to spend several weeks in her lab to learn how to analyze proteomic datasets. He will also work with the proteomic core at UC Davis run by Dr. Brett Phinney. Ryo will work with Dr. David Drubin, a pioneer in cytoskeletal biology. He will regularly attend in and present in David's lab meetings to gain direct feedback on his work. Additionally, Ryo will plan to work with biophysicists, including graduate student, Bob Cail, in the lab to better understand how to interrogate cytoskeletal form and function in ways he is currently not trained in as a cell biologist. Finally, Ryo will work with both me and Dr. Anne Brunet, a world-renowned aging scientist. Both of us are experts in the aging field and are directors of the Glenn Foundation, and thus will provide excellent support and training for Ryo in the field of aging biology. We will ensure that Ryo actively participates in Glenn Foundation events, such as the Bay Area Aging meeting.

One of the major limitations in studying cytoskeletal biology in *C. elegans* was the lack of tools available to interrogate the cytoskeleton. As a primarily genetic model organism, cell biological techniques are traditionally lacking. However, Ryo would not let this deter him, and his ambitious efforts have led to the development of tools that allowed him to measure cytoskeletal structure in a tissue-specific manner, and he has already published this work in an elaborate paper in *Molecular Biology of the Cell*. Ryo has also written a comprehensive review article on stress response in *Developmental Cell*. His work ethic is apparent in his diverse CV, featuring a large number of successful publications, acquisitions of grants and awards, and a unique exposure to management and teaching. While his many experiences in places like Burger King, Starbucks, Subway, and Armani Exchange may seem irrelevant, after visualizing Ryo's acumen and exceptional capacity to multi-task, I realize the value in his broad exposure. Ryo is constantly working on several projects and with many different people at the same time, and is incredibly able to consistently produce exceptional results without compromising any one thing. To

this end, Ryo already has one first-author publication and one first-author review in press, and two larger manuscripts, which he expect to be accepted by the end of this year: one in *Nature* and one in *Cell*, both of which were very favorably reviewed and revisions have already been submitted. In addition, he will be 2nd or 3rd author on three more manuscripts in which he has contributed greatly on as a collaborator both inside and outside of my lab. All of my lab members, including myself, am always impressed at his prolific capacity and it is truly remarkable to watch him work.

Finally, Ryo has performed a large number of intricate genetic screens in both *C. elegans* and in human tissue culture, which has yielded a large amount of highly interesting and valuable data. He was able to rapidly perform secondary and tertiary screens to create a well-established, hypothesis-driven proposal on understanding the mechanistic function of chromatin remodeling and lipid homeostasis on actin biology. The multiple screens were a heroic feat, and alone could have created an exciting proposal, but his remarkable qualities as a scientist are visible in his efforts to complete the screen prior to applying in order to create a more directed and clear hypothesis for his K99/R00 application. Ryo's work on cytoskeletal biology is very distinct from the other work that goes on in my lab. We have already agreed that all the data he has collected for his screen, as well as any data generated during the K99 portion of his grant, can be brought to his own laboratory when he acquires a faculty position. He is currently working on wrapping up several manuscripts in my laboratory: two on cytoskeletal biology, and four on the quality control of the endoplasmic reticulum, which he will be first author (or co-first author), and I will be senior author on. Beyond these publications, all future publications he will produce from his work will be his and he will be senior author on these manuscripts. We will maintain open dialogue in our future work to ensure that we do not have any competitive scientific overlap.

b. Training in teaching and mentoring

In my lab, Ryo has taken on an exceptional role in mentoring and leadership duties. Currently, he has two graduate students and three undergraduate students that he mentors. Throughout his short time in the laboratory, he has already mentored three rotation students, two of which have joined the lab, six undergraduate students, and four high school students. All of these students are still working in the lab, or have successfully transitioned into highly competitive programs. Ryo is an exceptional mentor and puts in immense time and effort into training his students, which is apparent in the great success that his students meet. Beyond his direct duties, Ryo also trains and helps his colleagues, having trained two new post-docs during their transitional period into the lab. Moreover, he builds meaningful and important collaborations within the lab, and helps his peers to succeed (see below for full list of trainees under Ryo in my lab). Ryo has made direct contributions to almost every project in my lab, working directly with graduate students and postdocs either in designing experiments, conducting experiments, or aiding in manuscript and grant writing. He also builds long-standing collaborations outside of the lab, and is working on very exciting projects with the laboratories of Drs. Amy Herr, Barbara Meyer, Roberto Zoncu, and Valerie Weaver.

I will continue to support his training in mentorship by assigning new students to him and giving him as many opportunities to train and support his colleagues. He also helps build the schedule of experiments for several technicians in the lab, and helps more senior students in designing manuscript outlines and formats, as well as posters and talks for presentations and meetings. Finally, Ryo meets monthly with the lab manager, Larry Joe, to build upon his already impressive mentorship and training experience to learn new techniques and skills to better manage a team. I allow Ryo to take part in both my lecture and laboratory courses. He acts as a teacher's assistant and aids in the development of my laboratory manual and course book, preparation for the classes, and in teaching. These skills will be useful for him as he transitions into a faculty position with teaching responsibilities. Ryo already has significant teaching experience, and this is apparent in his comfort and ease as he guides students through complex laboratory techniques and genetic methodology.

Name	Role with Ryo	Current Position
Gemma Araujo	B2B Summer Research Program	Student – UC, Berkeley
Raz Bar-Ziv	Postdoctoral Fellow in Training	EMBO Fellow – UC, Berkeley
Emily Beckman	CHORI Summer Research	Student – UC, Berkeley
Camila Benitez	Undergraduate Research Assistant	Technician – Dr. Andrew Dillin
Van Dinh	CHORI Summer Research	Student – Dr. Andrew Dillin
Stefan Homentcovshi	Undergraduate Research Assistant	Undergraduate Assistant – Dr. Andrew Dillin
Phil Frankino	Graduate Student	Graduate Student – Dr. Andrew Dillin
Gilberto Garcia	Graduate Student	Graduate Student – Dr. Andrew Dillin
Alex Jeronimo	CHORI Summer Research	Student – UC, Merced
Naame Kelet	Undergraduate Research Assistant	Undergraduate Assistant – Dr. Andrew Dillin

Jenessa Mendoza-Chavez	B2B Summer Research Program	Undergraduate Assistant – Dr. Andrew Dillin
Samira Monshietehadi	Research Technician in Training	Research Technician – Dr. Andrew Dillin
Joseph Paul	Rotation Student	Graduate Student – Dr. John Kuriyan
Marcela Preininger	Rotation Student	Graduate Student – Dr. Daniela Kaufer
Raymundo Sanchez	CHORI Summer Research	Student – UC, Irvine
Koning Shen	Postdoctoral Fellow in Training	JCC Fellow – Dr. Andrew Dillin
TingXuan Yang	Undergraduate Research Assistant	Undergraduate Assistant – Dr. Andrew Dillin

c. Research Support

Currently, my laboratory is funded both by the NIH and HHMI. Ryo's work on cytoskeletal biology outlined in the proposal will be supported by the following grants:

R01AG055891 (Dillin, PI) 04/01/2017-03/31/2022 NIH/NIA
The Collapse of Proteostasis during Aging is Mediated by Cytoskeletal Actin Functions The major goal of this project is to perform research towards the cytoskeleton's role in maintaining the overall health of the cell. Role: PI

-- (Dillin, PI) 09/01/2008-08/31/2024 Howard Hughes Medical Institute

Molecular Pathways of Aging The major goal of this project is to perform high risk, innovative research towards the understanding of aging and age-related diseases. Role: PI

d. Direct supervision and mentoring

Throughout the 15 years as a principal investigator, I have trained and mentored 30 postdocs and 13 graduate students, and currently have 11 postdocs and 6 graduate students in the lab. All of my trainees have been exceptionally successful at obtaining positions both in academic institutions and in biomedical companies. I have had several post-docs who have received major transitional funding awards, including Dr. Peter Douglas who received the Pathway to Independence Award in 2013 and successfully transitioned into a tenure-track position at UT Southwestern, and Dr. Celine Riera who received the Pathway Initiator Award from the American Diabetes Association and successfully transitioned into a tenure-track position at CedarsSinai/UCLA. My additional noteworthy postdoctoral trainees are listed below.

Ryo formally presents his research every six months in our lab meetings, and his collaborative work with his graduate students, Phil Frankino and Gilberto Garcia, are also independently presented every six months. This gives me several formal opportunities to evaluate the progress of the work where there is also feedback and input from the rest of the lab. More importantly, I meet with him after every lab meeting to further discuss future directions on his work, his students' works, and his success in mentoring and training his students into success. We also have formal one-on-one meetings every month, which is a dedicated one hour block of time where we discuss research progress, career goals, grant applications, and preparation for publications and job searches. In addition to these concrete meetings, I meet with Ryo informally every few days. Therefore, I have ample opportunities for mentoring and guiding Ryo into a successful faculty position.

In addition to my own mentorship and the mentoring he will receive formally from his committee, Ryo will gain mentorship from the many principal investigators that come through my lab for sabbatical research. Currently, Ryo works very closely with Drs. Jens Brüning from the Max Planck Institute and Reinhard Fischer from the Karlsruhe Institute of Technology (KIT). Both Jens and Reinhard are helping Ryo in preparing for the faculty search, and he receives significant feedback in drafting the necessary documents for applications. These experiences, and experiences with future visiting professors, are invaluable in his career as they provide many perspectives from different institutions and vastly different labs on how to be a successful independent faculty member. He is also working on collaborative manuscripts with Drs. Amy Herr, Roberto Zoncu, and Barbara Meyer, who provide great mentorship. Amy is an exceptional bioengineer, and provides a scientific and experimental perspective unique for Ryo, and they are working on an exciting project in creating a novel method for interrogation of cytoskeletal quality with single cell resolution. Ryo works with Barbara on a project understanding how X chromatin form and structure can alter stress response and lifespan, and this work is a fantastic gateway to his own work on how chromatin structure can affect cytoskeletal quality. Ryo has also independently started a collaboration with Roberto to strengthen his work on lysosomal recycling of amino acids on ER quality, as Roberto is an expert in biochemistry and lysosomal biology. Ryo will be a significant contributor in these manuscripts, which are expected to be completed by the end of 2019. Moreover, Amy, Barbara, and Roberto all offer their full support in mentoring and guiding Ryo throughout the K99/R00. They have each written

very strong letters of supports in this effort (Barbara as a co-sponsor, Amy as a collaborator/advisory committee member, and Roberto as a reference letter), which highlight how his scientific prowess have impressed them, but also their commitment to training him into a stronger, more well-rounded scientist. The Herr lab will be a critical resource for him to carry out his biochemical assays to interrogate cytoskeletal quality, while the Meyer lab will provide support for him to perform assays to assess chromatin state and gene expression, including ATAC-seq, CHIP-seq, and RNA-seq.

Name	Affiliation	Title
Hugo Aguilaniu, Ph.D.	Ecole Normale Supérieure de Lyon, France	Group Leader/ Principal Investigator
Nathan Baird, Ph.D.	Illumina, San Diego, CA	Senior Scientist
Ehud Cohen, Ph.D.	The Hebrew University, Jerusalem	Professor
Joseph Daniele, Ph.D.	MD Anderson, Houston, TX	Institute Researcher
Peter Douglas, Ph.D.	UT Southwestern Medical Center	Assistant Professor
Hyun-eui Kim, Ph.D.	McGovern Medical School at UTHealth	Assistant Professor
William Mair, Ph.D.	Harvard University	Assistant Professor
Carsten Merkwirth	Ferring Research Institute, San Diego, CA	Scientist
Johan Paulsson, Ph.D.	Novo Nordisk A.S., Denmark	Principal Scientist
Celine Riera, Ph.D.	Cedars-Sinai/UCLA, Los Angeles, California	Assistant Professor
Ophir Shalem, Ph.D.	University of Pennsylvania	Assistant Professor
Kristan Steffen, Ph.D.	Grail, San Francisco, CA	Scientist
Rebecca Taylor, Ph.D.	MRC, Cambridge University	Group Leader/ Principal Investigator
Ye Tian, Ph.D.	Chinese Academy of Sciences, Beijing, China	Assistant Professor
David Vilchez, Ph.D.	CECAD Research Center University of Cologne, Germany	Group Leader/ Principal Investigator

e. Communication

Ryo has already published several high impact research studies, and it is clear through our work together that he is an excellent writer. I allow Ryo to independently write his manuscripts and grants, and the first draft presented to me is always a fully completed document. To hone his writing skills further, we work together on improving manuscripts, and we work both electronically and in person as I go over how to strengthen his work. To date, Ryo has published 7 first-author manuscripts in Liza's lab, we have published two manuscripts in my lab: one primary literature and one review article, and have successfully acquired an R01, together. Currently, Ryo is working with multiple members of the lab on various projects, and has taken a major role in preparing their manuscripts and grants. These works have created numerous high-impact papers: one revision that was submitted to *Nature*, another revision submitted to *Cell*, both of which have been reviewed very favorably and expect to be published within the year. He will also be submitting another manuscript to *Nature Metabolism* by the end of the year. He has also helped several postdocs in the lab successfully acquire fellowships. I continue to encourage Ryo to work with both graduate students and postdocs in their writing so that he can acquire mentorship skills and learn various writing styles.

I will encourage Ryo to give talks at several conferences and seminars during the K99 phase. He has already given a formal seminar at UC Berkeley, and has presented his early work both at NIA and HHMI meetings, and informally on posters at meetings in the Bay Area, including the Bay Area Worm Meeting and Bay Area Aging Meeting. Ryo plans to present his more developed work at Keystone Symposia, Cold Spring Harbor Laboratory Meetings, Gordon Research Conferences, and in several bay area meetings. I will also send Ryo to the Molecular and Cell Biology and Genetics departmental retreats in 2019 and 2020.

f. Transition into independence

During the K99 phase of the grant, Ryo will meet with the advisory committee every 6 months to discuss research progress. At the culmination of Year 1, we will assess whether Ryo is prepared to step into the faculty search process. I fully expect that Ryo will have an impressive CV and necessary skillsets to successfully begin his faculty search at the beginning of Year 2. During this process, I will work with Ryo through the application, interview, and negotiation process. As an exceptionally eager and ambitious scientist, Ryo has already drafted several research statements that I found impressive and well thought out. I will work with Ryo to develop these drafts as his project develops, and mentor him on how to cater applications to institutions to be a more competitive applicant. The members of his committee will also contribute as required.

I fully expect Ryo to be offered several faculty positions in Year 2 of his K99. During this phase, I will assist in his negotiation process. My lab manager, Larry Joe, will aid in his equipment negotiation and interviewing prospective hires as he builds his lab. While Ryo has great skills in negotiation and management from his restaurant and retail management experiences, Larry will help Ryo hone these skills for the laboratory space. He is continuously working with Larry to learn and build these skills, and has regular one on one meetings for this purpose. During the R00 phase, I will assist Ryo in any way I can, and welcome Ryo to come to me for feedback on grant applications, papers, scientific direction, teaching, and career advice. I will also push for Ryo to give talks in meetings I organize, and help in staff acquisition by forwarding candidates that I believe will be a great fit in his lab.

g. Final remarks

It is clear through Ryo's exceptional and diverse CV that he is an excellent scientist with extraordinary work ethic. I have supervised many scientists at various stages of their career, and I have never seen someone who works as tirelessly and with dedicated passion as Ryo does. It is very clear why he had so many publications during his graduate work, and I fully expect that he will have just as many successful publications during his postdoc in my lab. He has already published a very thorough review and primary literature article, both of which have set the stage for his current work and the work proposed in this grant. He also has two first-author articles coming up shortly: one currently under review in *Cell*, which I have faith will be reviewed very favorably, and another under revision for *Nature*, which I fully expect to go through without any issues. He will be a significant contributor on two papers in collaborating labs with Drs. Amy Herr and Barbara Meyer, and is currently wrapping up several other publications with other members of the lab. His ability to multi-task and work on so many different things without compromising work on any single thing is remarkable. Ryo is heavily involved in many different projects with significantly different focuses, and he does a great job keeping everything on track. Moreover, Ryo constantly has great feedback for every project ongoing in my lab, and is one of the most vocal members of my lab during lab meetings or discussions of any kind. He is also the first to volunteer in helping all other members of the lab with experiments, technical work, writing and editing of manuscripts and grants, and anything else someone could require help with at any given moment. Ryo also goes above and beyond to ensure that the lab runs smoothly, being the first to volunteer in making reagents, cleaning, dealing with trash and hazardous resources, ordering, or anything that could possibly help in any way.

In addition to his mastery of multi-tasking, being a great scientist, and generally a fantastic lab citizen, Ryo is exceptionally humble and kind. He always volunteers to host and organize lab outings and parties, including baby showers, going away parties, and team-building exercises. Ryo truly goes above and beyond to make sure that every member of the lab feels welcome and part of the team. Through all this, it is clear that Ryo will make an exceptional faculty member and run a highly successful lab with a strong team.

Sincerely,

[redacted]

Andrew Dillin, Ph.D.
Thomas and Stacey Siebel Distinguished Chair in Stem Cell Research
Howard Hughes Medical Investigator
Professor, Department of Molecular and Cellular Biology

(II) Co-Sponsor Information

I write this letter to show my enthusiastic support and commitment to serve as a co-mentor for Ryo Higuchi-Sanabria's K99/R00 application. I have known Andy Dillin and worked closely with him for several years and believe his lab is a great environment for mentoring postdocs to become high accomplished faculty members. I have collaborated directly with Ryo for over a year to complete a research project in my lab demonstrating the impact of chromosome structure on stress responses and lifespan in *C. elegans*. I believe that Ryo is a strong candidate for the K99 application. Through our collaboration, it is apparent that Ryo is highly creative, extraordinarily motivated, fearless in developing new technical approaches, and prolific in his research accomplishments.

My lab focuses on chromosome structure and chromosome dynamics during development. We study the molecular mechanism governing sex determination in *C. elegans*, which we showed utilizes a series of X-signal elements to communicate X-chromosome dose and autosomal-signal elements to communicate ploidy. We also study X-chromosome dosage compensation, in which a condensin complex binds specifically to X chromosomes of hermaphrodites to establish a unique chromosome architecture, modify chromatin composition by catalyzing enrichment of histone modification H4K20me1, and repress transcription. We recently demonstrated that deleting selective binding sites for the condensin complex disrupts higher-order X-chromosome structure and causes the X to have the structure of an autosome. In collaboration with Ryo we showed that abrogating the X structure reduces thermotolerance, accelerates aging, and shortens lifespan, implicating chromosome architecture in regulating stress responses and aging.

In my capacity as a co-mentor, Ryo and I will have monthly meetings to discuss his progress, with more frequent meetings during times when we will be working on ChIP-seq, ATAC-seq, and RNAseq experiments to pursue his K99 goals. My lab is highly experienced and well equipped to perform ChIP-seq, ATAC-seq, and RNAseq in *C. elegans*, and my lab members will train him to become proficient in both the technical work and post-sequencing analysis. Currently, all members of my lab have been individually trained to perform their own bioinformatics analyses, using various different software, including bowtie2, SAMtools, deepTools, and macs2 for ATAC-seq and CHIP-seq, and tophat and cufflinks for RNAseq. Therefore, I have full confidence that we will be able to properly train and equip Ryo to perform the experiments in his proposal.

During his training, I will have Ryo attend the python and R bootcamps taught at UC Berkeley, which are offered to postdocs and graduate students. This will provide him a great introduction into coding and computational work required for his post-sequencing analyses. In my lab, he will work directly with Drs. Erika Anderson, Nick Fuda, and Kristina Krassovsky, who regularly perform the types of experiments laid out in his proposal. I fully expect Ryo to be independent in post-sequence analyses within his first year in my lab. After this initial year of training, my lab and I will continue to provide feedback and support throughout the K99 portion of the grant. This will be in the form of direct in-lab mentoring, as well as experimental and scientific feedback during lab meetings, which Ryo will attend and present regularly. The members of my lab are experts in dissecting chromatin and chromosome architecture and the functional consequence of chromatin structure and state. Therefore, we are scientifically well-equipped to guide Ryo in understanding the novel and exciting finding that chromatin architecture and chromatin remodeling may alter actin organization and function throughout the aging process.

I have extensive experience in mentoring Ph.D. students and post-doctoral fellows and feel certain that my mentoring experience will aid Ryo in his research and career. I directed my department's Ph.D. program for 6 years and was PI of the NIH Graduate Genetics Training Grant. I serve on advisory boards for graduate and postdoctoral training: SAB of the Helen Hay Whitney Foundation for Postdoctoral Fellowships, Advisory Board for the Cold Spring Harbor Ph.D. Program, Senior Fellow of the Miller Institute at Berkeley to mentor postdoctoral fellows. Of my 38 Ph.D. students and 37 postdoctoral fellows, virtually all have prominent positions as faculty, senior scientists in industry, or scientific writers.

I am confident that the research Ryo proposes for the K99 award will be highly successful and that he will transition easily into a faculty member at a prestigious university. In short, Ryo is an exceptional scientist--a pure gem, who is highly deserving of a K99 award.

Sincerely,

Barbara J. Meyer, Ph.D.
Investigator, Howard Hughes Medical Institute
Professor of Genetics, Genomics, and Development at U.C. Berkeley
Adjunct Professor, Department of Biochemistry and Biophysics, U.C.S.F. School of Medicine

STANFORD UNIVERSITY
DEPARTMENT OF GENETICS

Anne Brunet, PhD
Email: [redacted]
Tel: [redacted]
Web: www.stanford.edu/group/brunet/

June 18, 2019

Dear K99 Review Committee:

I enthusiastically write this letter to show my complete support and commitment to serve both as a mentor on Ryo Higuchi-Sanabria's advisory committee, and a collaborator. I have known Andy Dillin and worked closely with him for several years, and believe his lab is a great environment for tailoring postdocs into faculty members, especially in the fields of aging and stress biology. Ryo and the rest of the members of his lab regularly attend the Bay Area Aging Meeting (BAAM), which is an annual meeting sponsored by the Glenn Foundation for Medical research, which I host along with Drs. Danica Chen, Pankaj Kapahi, and Hao Li. BAAMs are focused on research in the diverse biology of aging and age-related diseases where scientists can network amongst participants, and Ryo and the rest of the Dillin lab are active and eager participants.

My lab focuses on studying the complex phenomenon of aging and age-related diseases, including cardiovascular disease, cancer, diabetes, and Alzheimer's disease. Specifically, my lab focuses on how the external environment can impact and influence the aging process, using multiple organisms: *C. elegans* and African killifish for their high-throughput abilities, and then translate our findings to mammals using mice and human cells. We study a diverse range of topics, including the impact of diet and metabolism in aging, maintenance of regenerative stem cells during aging, cognitive aging of the brain, and perhaps most relevant to Ryo's work, epigenetic integration of external aging signals. We are highly interested in how chromatin states change with age and how environment stimuli, such as food, sex, pathogens, etc. can influence chromatin state and alter lifespan. Considering this expertise, I believe my lab would be a great resource as he continues to study the impact of chromatin state and chromatin modifiers on cytoskeletal quality, especially during the aging process.

Ryo and I have agreed to meet every three months with more frequent meetings when needed. Moreover, Ryo will present regularly (at least every 6 months) during my lab's group meeting to get directed feedback from all members of my lab. My lab is also well-equipped to perform RNA-seq, ChIP-seq, and ATAC-seq, all of which we offer our full support as he trains to become proficient in these techniques. I believe that my lab and Andy's lab together provide a fantastic training and mentoring capacity for Ryo to develop into a highly proficient scientist and contributor to the aging community.

Sincerely,

[redacted]

Anne Brunet, Ph.D.
Michele and Timothy Barakett Professor of Genetics

UNIVERSITY OF CALIFORNIA, BERKELEY

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OFFICE OF THE CHAIR
DEPARTMENT OF MOLECULAR AND CELL BIOLOGY
COLLEGE OF LETTERS AND SCIENCE
[redacted]

PHONE: [redacted]
EMAIL: [redacted]

November 16, 2018

Dear K99/R00 review committee,

I would be happy to assist Ryo Higuchi-Sanabria as part of his advisory committee during his K99 award.

I have known Andy Dillin for many years and believe that his lab is an excellent environment for postdoctoral training. The past postdocs in his lab have all moved on to great careers, and a large number of them have successfully transitioned into tenure-track faculty positions. Ryo will receive excellent training and mentorship throughout his K99 in Andy's lab, with the help of his advisory team.

My lab investigates the molecular mechanisms underlying actin dynamics, particularly in relationship with membrane trafficking events. We use diverse approaches including state-of-the-art live-cell imaging, genome-wide functional analyses, genetics, and biochemistry to answer our questions. My lab has a long-standing interest in actin cytoskeletal dynamics and the mechanisms involved in how the cytoskeleton exerts forces to move membranes in a cell. Our extensive expertise in actin biology is sure to be of use in Ryo's proposal in understanding the form and function of actin throughout the aging process. I will provide scientific advice as his mentor throughout his K99 research, and the members of my lab can provide experimental and technical support to carry out his research. Since Ryo's experience is mostly in live-cell work, I believe that my lab's expertise in *in vitro* assays to study cytoskeletal form and function will benefit him greatly and add to his skillsets.

As part of his mentorship committee, I will work to evaluate his scientific and career progression, as well as provide feedback, support, and suggestions for his growth and development. I will participate in committee meetings and meet with Ryo minimally every 6-9 months and more frequently as needed as his project develops. I will introduce him to postdocs and graduate students in my lab to aid in his mentorship and growth. Ryo has already had very successful interactions with my lab and worked with a former postdoc, Daphné Dambournet, in developing a project in collaboration with the Herr lab to biochemically interrogate cytoskeletal quality with single-cell resolution. This partnership gives me great confidence in his capacity to work productively with my lab.

Sincerely,

[redacted]

David G. Drubin
Professor and Co-Chair, Department of Molecular and Cell Biology

Howard Hughes Medical Institute

Michael B. Eisen, Ph.D.
[redacted]

December 10, 2018

Dear K99 Review committee,

I am writing to express my enthusiastic support for the application of Ryo Higuchi-Sanabria for a K99, and commitment to serve both as a mentor on his advisory committee and collaborator. I have known his PI Andy Dillin for many years and have worked closely with people in his lab in many contexts. He is a wonderful scientist and colleague, and fantastic mentor whose lab provides the perfect environment for transforming postdocs into PIs. I have met Ryo and discussed his project, and heard nothing but praise about his intellect, skills and work ethic, and I believe that he is a strong candidate for the K99.

My lab studies the biochemical mechanisms involved in gene expression, with a strong focus in understanding the regulation and function of enhancer elements. We work on many different processes surrounding enhancer identity, function, and evolution, using a combination of genetics, genomics, molecular biology and imaging, knitted together with computation. I believe that my lab has the ideal expertise to guide Ryo in dissecting the transcriptional control of chromatin modifiers and dissecting chromatin states that govern cytoskeletal regulation. We have pioneered genomic methods, and are experts in the application of ATAC-seq and similar techniques, and can pass this knowledge on to Ryo as he develops related methods in worms during his proposal.

Ryo and I have agreed to meet at least twice a year, with more frequent meetings during times when he will be working on developing his ChIP-seq or ATAC-seq platforms in his lab. My lab is well equipped to perform these experiments and my lab members will train him to become proficient in both the technical work and post-sequencing analysis. I will aid in Ryo's development in his science and his career, and feel that with the combination of his skills and the organization of his mentoring committee and application, he is well prepared to succeed in the proposed experiments and transition into a faculty position.

Sincerely,

[redacted]

Michael B. Eisen, Ph.D.
Department of Molecular and Cell Biology
Howard Hughes Medical Institute
University of California, Berkeley

[redacted]

Dear esteemed colleagues & NIH K99 selection committee,

I write with enthusiastic support for Ryo Higuchi-Sanabria, Ph.D. and his NIH K99 proposal. We have collaborated for ~2 years on refining and applying nascent single-cell protein measurement technologies – in this collaboration, we are strongly guided by Dr. Higuchi-Sanabria's biological insights and vision. What a perfect fit between this project, this promising investigator, and the NIH K99/R00 funding mechanism – given the program's stated goal to "increase and maintain a strong cohort of new and talented, NIH-supported, independent investigators." Successful completion of Dr. Higuchi-Sanabria's proposed research in identifying novel mechanisms that govern the regulation of the actin cytoskeleton is unique and exciting in the field of aging. However, as his proposal highlights, precise and accurate tools to interrogate cytoskeletal health are lacking. While Ryo has created novel tools to qualitatively interrogate cytoskeletal health in *C. elegans*, the advent of tools to quantitatively analyze cytoskeletal health would be a tremendous boost to the field of aging research. Thus, Dr. Higuchi-Sanabria approached my lab to partner in designing, developing, and applying single-cell resolution immunoblotting to identify the type and abundance of actin present.

Given the tremendous advances in single-cell genomics and transcriptomics, my lab has focused on new tools for measuring proteins in single cells: we have contributed a suite of single-cell immunoblot tools, built on microfluidic technologies and optimized for the specific properties of cells and biological molecules in question. Our single-cell immunoblots are capable of measuring cell-to-cell variation in protein-mediated functions using a microfluidically instrumented microscope slide. Cells are isolated in microwells, then lysate from each cell is subjected to any one of set of electrophoretic modalities (protein sizing, native electrophoresis, gradient electrophoresis, isoelectric focusing, etc.) and blotted onto a light-activated polymer matrix. The probing step of each immunoblot uses common immunoreagents. We have designed assays for scrutiny of neural stem cell differentiation (Hughes, Spelke, et al., *Nature Methods,* 2014), multiplexed protein analysis of circulating tumor cells (Sinkala et al., *Nature Communications*, 2017), subcellular localization of a range of dynamic protein targets including NF-kb (Yamauchi, et al., *NPJ Microsystems & Nanoengineering*), HER2+ breast cancer classifications including truncated HER2 isoforms and signaling proteins (Kang, et al., *Nature Precision Oncology,* 2018), and ascertaining protein expression profiles in 2-cell and 4-cell murine embryos to understand early lineage bias or plasticity (Rosas, et al., in review at *Science Advances*). I mention a handful of our studies here, mostly focused on biological and biomedical applications, to highlight the diversity of areas where single-cell resolution & high selectivity protein targets are needed.

Specific to Dr. Higuchi-Sanabria's proposed studies: we are designing and now optimizing the use a sequence of immunoblots – all applied to the same cell, but under different buffer conditions -- to distinguish between G-actin (a monomer) and F-actin (a polydisperse population of G-actin multimers) species. We are aiming to offer the first tool to be able to robustly quantify F and G actin with single-cell resolution. We are adapting a serial lysis technique (differential detergent fractionation) developed in our lab for application to single cells, as a way to isolate and analyze cellular organelles. As mentioned, we have reported on successful immunoblotting of cytoplasmic protein targets and nuclear protein targets from the same cell in a unique 'bi-directional electrophoresis' separation stage (i.e., cytoplasm lysed, cytoplasmic proteins subjected to immunoblot with nucleus intact; then nucleus lysed and nuclear lysate subjected to an immunoblot). Moreover, our preliminary work gives us full confidence that we can optimize this technique towards the analysis of actin, as described in Dr. Higuchi-Sanabria's K99 application. If all goes accordingly to plan, I estimate our first manuscript submission this calendar year.

As you can see, we have a good complementary approach to Dr. Higuchi-Sanabria's interests and a solid working relationship. Our goals are to accelerate development of analytical methods, streamline sample preparation strategies, and improve molecular validation studies – what better motivation than a budding scientist in the challenging area of G- and F-actin biology! If Dr. Higuchi-Sanabria is so fortunate to be supported by NIH in this way (which I heartily endorse!), my lab's expertise will be at hand to aid Dr. Higuchi-Sanabria and continue our collaboration. Currently, two outstanding researchers in my laboratory are actively collaborating with Dr. Higuchi-Sanabria on this intriguing question – looking forward, it would be thrilling to see Dr. Higuchi-Sanabria's career launch around this timely, important, interdisciplinary, and challenging topic. He's going to make important contributions throughout his career – I have no doubt – and I do hope they are in this area.

Sincerely,

[redacted]

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UC DAVIS GENOME CENTER
PROTEOMICS CORE FACILITY
[redacted]
[redacted]

[redacted]
[redacted]

November 26, 2018

Dear K99 selection committee,

I am very pleased to write a letter on behalf of Ryo Higuchi-Sanabria in support of his K99 proposal. The UC Davis Genome Center's Technology Service Cores have been established to enable researchers, such as Ryo, to carry out technology-intensive research projects. The novel approaches described in the proposed research in finding unique molecular interactors of actin in a tissue-specific manner is very exciting, and we certainly will be able to assist in the research.

We have more than 2 years of experience in analyzing TMT samples using our new Thermo Fusion Lumos mass spectrometer and have been very successful in measuring and detecting differentially expressed proteins using the SPS MS3 method. We have a large amount of experience analyzing both single TMT and multiple spanning TMT experiments using R. In conjunction with the bioinformatics core here at UC David Genome Center, we can assist Ryo with the complex analysis and experiment designs that are a part of his proposal.
The UC Davis Genome Center utilizes both experimental and computational approaches to address scientific questions. We are located in a new research building with state-of-the-art computational and laboratory facilities. The Center has 15 research faculty and five technology cores that serve the entire campus and off-campus collaborators. We have made major investments in state-of-the-art instrumentation and recruitment of experienced staff.

I currently head the proteomic core, which has a staff of four experienced protein and mass spectrometry scientists. For high throughput identification and characterization of proteins and peptides, the proteomics core has at its disposal several state of the art LC-MS/MS systems including two HPLC (300 Bar) and nano-UPLC (1200 Bar) and a new (2016 Thermo Scientific Fusion Lumos with ETD mass spectrometer connected to an Ultimate 3000 nano UPLC. We also have Thermo monitoring targeted protemics experiments. My laboratory can also provide amino acid analysis using our three Hitachi amino acid analyzers and N-terminal sequencing using our two N-terminal sequencers if needed. In addition, the Genome Center has 2 medium-sized computer clusters and one large 400 core cluster that we can access for data analysis.

The UC David Genome Center also has additional core facilities, including bioinformatics and metabolomics to provide support for Ryo's research. We can perform all of Ryo's mass-spec work and the bioinformatics core can assist in the organization and interpretation of the complex data sets that may be obtained from his studies. We are pleased to provide access to these state-of-the-art facilities that will enable Ryo to pursue this important research.

[redacted]

Brett S. Phinney, Ph.D.

STANFORD UNIVERSITY
DEPARTMENTS OF GENETICS, BIOLOGY, & CHEMISTRY (by courtesy)
[redacted]

ALICE Y. TING
PROFESSOR

TEL. [redacted]

November 14, 2018

Dear K99 Selection Committee:
I am pleased to write this letter to acknowledge my participation on Ryo Higuchi-Sanabria's mentorship committee, and to serve as a collaborator in his efforts to identify the molecular interactome of the actin cytoskeleton.
Ryo has contacted me due to his interest in utilizing our most recent proximity-based protein labeling tool that our laboratory has developed. My lab is well-versed in detecting and mapping spatial organization of cellular components with nanometer precision and temporal acuity. We map molecular interactomes on a sub-cellular scale, and elucidate the circuits, signaling, and communication between these molecules. We have engineered many intricate advancements in enzyme-mediated proximity biotinylation, the most recent, miniTurbo, being one that Ryo plans to use in his studies. This mutant of biotin ligase catalyzes proximity labeling at much greater efficiency, which will allow Ryo to gain very specific temporal resolution of biotin labeling without biotin supplementation, as the biotin acquired by the bacteria is sufficient in worms.
This technology is ideally suited to help Ryo in identifying cytoskeletal interactors in a tissue-specific manner as described in his K99 application. I believe that my expertise will aid in the development, troubleshooting, and validation of his assay. I have already provided him direct feedback to his proposal in an earlier conference where I met him to incorporate a cytoplasmic version of his protein to eliminate any non-specific cytoplasmic noise from his datasets.
The members of my lab can also train him in proper processing of his samples and with analysis of his proteomic data. I have full confidence in our collaboration, and will meet with him every 6-9 months – or more frequently as needed – as the work progresses. I will also participate in his committee meetings to discuss Ryo's data and provide feedback on his career progression.
Sincerely,

[redacted]

Alice Ting

Institutional Environment

A majority of the proposed experiments will be performed in the laboratories of Dr. Andrew Dillin in the Li Ka Shing Center and Dr. Barbara Meyer in the Koshland building at the University of California, Berkeley. UC Berkeley is a well-established and leading research institute across the country, and is heralded as one of the top public universities. The university currently employs 21 Howard Hughes Medical Institute Investigators, 33 MacArthur fellows, 7 nobel laureates, and 144 members of the National Academy of Sciences.

Scientifically, Dr. Dillin's laboratory investigates mechanistic pathways in quality control of the mitochondria and ER, the cytoplasmic stress response, and the role of neurons and glia in these pathways. The mastery of many fields provides me the opportunity to expand my knowledge on stress and aging biology, while also having the freedom to create my own niche in cytoskeletal biology. Experimentally, the Dillin lab has expertise in many different techniques in multiple model systems. Originally trained in cell biology, I have spent my early postdoc working on mastering genetics with the great help of the lab, and plan to expand my expertise into biochemical and computational skills through this proposal. I will work with great computational experts in my lab, including postdoctoral fellows, Erica Moehle and Raz Bar-Ziv, who perform large-scale data analysis on a daily basis. The Meyer lab investigates molecular networks that control chromosome behavior during sexual fate, X chromosome dosage compensation, chromosome cohesion during replication, and chromosome structure during meiosis. Experimentally, the Meyer lab has expertise in interrogating chromosome structure and architecture using genetic, molecular, and computational strategies. I will work with Dr. Meyer and her many experienced members, including Drs. Erika Anderson, Nick Fuda, Kristina Krassovsky, Satoru Uzawa, and Ed Ralston. The Meyer lab will play a critical role in guiding the CHIP-seq, ATAC-seq, and RNA-seq elements of the proposal to investigate the role of chromatin structure in cytoskeletal regulation.

Outside of the lab, Drs. Dillin and Meyer participate in many leading conferences and meetings around the world, which he invites senior members of his lab to. I plan to join Dr. Dillin in several meetings to learn presentation skills, network, and learn how the structure of conference organization works. Beyond the unmatched scientific opportunity, both the Dillin lab and UC Berkeley provide a unique opportunity to continue to explore my interests in scientific teaching both in and out of the lab. Dr. Dillin has allowed me the privilege to work with many high school, undergraduate, and graduate students, as well as mentoring and training new technicians and postdocs. This is invaluable for when I have my own lab. Finally, Dr. Dillin runs both a lecture series and a lab series for genetics courses, of which he allows me to continue to participate in. I will continue to increase my teaching skills by participating in running his laboratory course, and will design and run the lab course in subsequent semesters.

In addition, UC Berkeley houses many phenomenal faculty, including Dr. David Drubin, an expert on cytoskeletal dynamics, and Dr. Michael Eisen, a leader in chromatin biology, both of whom are on my advisory committee and have welcomed me into their labs to work directly with the members. In addition to my committee, Berkeley has Dr. Ke Xu who developed unique quantitative methods for leading edge super-resolution microscopy, Randy Schekman, a prominent figure in vesicular trafficking and a founder of the widely successful open-access journal, *eLife*, and many other prominent scientists who will provide an unlimited wealth of mentoring, training, and invaluable collaboration. The Dillin lab shares a floor with Drs. Randy Scheckman, Robert Tjian, Xavier Darzacq, Daniela Kaufer, and Dirk Hockemeyer, all of whom share lab space, reagents, and equipment, and all of whom directly participate in my mentorship and learning.

UC Berkeley's imaging core hosts an impressive array of microscopes with powerful microscopes including the LSM 710 and a wide array of well-developed imaging techniques, such as CellASIC microfluidic devices and SIM/STORM super-resolution microscopy, which provide multiple training opportunities far beyond my already in-depth imaging experience. These will serve a critical role in the imaging strategies described in the research plan. Other integral core facilities to propel my research is the computational Genomics Resource Laboratory and Mass Spectrometry cores that will provide direct technical expertise and training to extract enormous amounts of essential data from transcriptome and mass-spec analysis proposed in the research plan. It is clear that my training at the Dillin lab will direct me to a career as an independent investigator and teaching faculty at a leading university.

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DONALD C. RIO
CO-CHAIR, DEPARTMENT OF MOLECULAR AND CELL BIOLOGY
PROFESSOR
DIVISION OF BIOCHEMISTRY, BIOPHYSICS AND STRUCTURAL BIOLOGY
[redacted]

TEL:
FAX:
E-MAIL:
E-MAIL:
[redacted]

Nov 21, 2018

To the K99/R00 review committee,

Dr. Ryo Higuchi-Sanabria is currently appointed with the University of California, Berkeley as a postdoctoral scholar in the Department of Molecular and Cell Biology (MCB). This letter is to confirm that the MCB department supports Ryo's NIH Pathway to Independence proposal, and his full-time appointment is anticipated to be maintained throughout the duration of the K99 postdoctoral phase. Continuation of his salary and position is not contingent upon receiving this award. Ryo will dedicate 100% of his time for research. Moreover, he will continue to participate in scientific meetings and voluntary mentorship and teaching opportunities to the extent that these will further his research and career development for an independent faculty position. Ryo works in the Dillin lab, where he has access to state-of-the-art equipment, including microscopes, computers, and other necessary laboratory equipment required for him to carry out his proposed research. Moreover, he is in close proximity with many other highly successful laboratories, sharing a laboratory space and equipment with the laboratories of Dr. Robert Tjian, Dr. Xavier Darzacq, Dr. Randy Schekman, Dr. Daniela Kaufer, and Dr. Dirk Hockemeyer. He also has many resources at UC Berkeley's campus, including the Functional Genomics Laboratory, next-generation sequencing facility that can aid in all of his sequencing needs starting from library preparation all the way to data analysis. Similarly, there is a Proteomics/Mass Spectrometry core affiliated with QB3 to provide a wide range of proteomics services. Finally, there is a large Molecular Imaging Center equipped with powerful microscopes including confocal and multi-photon, spinning disk, light-sheet, and high-resolution microscopy.

In addition to these many technical resources, Ryo will have many opportunities to interact with trainees and faculty at our off-campus departmental retreat, regular seminars, journal clubs, sponsored socials, and many local Bay Area scientific meetings. Finally, MCB post-docs can attend talks of prospective faculty at UC Berkeley to gain insight on the interview process. Upon completion of his postdoctoral work, Ryo will be pursuing an independent, tenure-track academic research position to continue his exciting work on actin cytoskeletal form, function, and regulation during the aging process. His continued postdoctoral training during his K99 phase at UC Berkeley will expand his scientific and experimental versatility. Professor Dillin has a strong track record for training and preparing postdoctoral fellows into tenure-track faculty positions, and his added guidance from collaborators and his committee, including Drs. Amy Herr, Barbara Meyer, David Drubin, and Robert Tjian at Berkeley, as well as others from outside of Berkeley, will add to his already exemplary training in his lab.

Sincerely,

[redacted]

Donald Rio
Professor and Co-Chair, Department of Molecular and Cell Biology
Professor of Biochemistry, Biophysics, and Structural Biology

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No
Other Requested information	

Resource Sharing Plan

UC Berkeley is committed to ensuring that biomedical research resources developed with NIH funding, such as the nematode strains synthesized in this study, are made readily available to the research community in compliance with the NIH Grants Policy Statement. We will adhere to these requirements by making all nematode strains synthesized in this study available through the CGC, while plasmids and expression vectors will be made available through Addgene. UC Berkeley also has a streamlined, one page MTA that is very unrestrictive, and allows for easy transferring of research resources to the research community for non-commercial research purposes. This will allow us to easily share resources that have not yet been deposited to CGC, Addgene, or equivalent. In line with UC Berkeley recommendations, I will avoid signing MTAs from outside parties that include royalties, product reach-through, or patents that may prevent subsequent access to research resources arising from these projects.

Should UC Berkeley pursue intellectual property protection and commercial licensing of certain research resources pursuant to the Bayh-Dole Act, UC Berkeley retains the right in its commercial license agreements to provide materials to the research community for non-commercial research purposes. UC Berkeley typically licenses research resources non-exclusively for commercial purposes unless there is an overriding reason to license it exclusively. In any exclusive commercial license, however, UC Berkeley will retain rights to provide the research resource to the research community.

UC Berkeley also utilizes central databanks and repositories such as The Jackson Laboratory and the American Type Culture Collection (ATCC), as necessary, to assure the availability of research resources to the research community when it is not feasible to maintain a research resource in-house or there is an overwhelming demand for a research resource.

Data Sharing Plan

All published data will be provided through the published journal in both raw and processed forms. For unpublished data, presentation of data generated from these projects will be shared at conferences and meetings. Our budget requests a specific allocation of funds for traveling, and I anticipate that I will be presenting my work in at least two conferences per year. Meetings at which I plan to attend include Keystone, Cold Spring Harbor, and Gordon meetings focused on aging and cytoskeletal biology. The results of this work is planned to be published in peer-reviewed journals, which allow public access per NIH standards. At publication, we will provide unrestricted access to all raw and processed datasets, including RNA-seq, proteomic mass spec, ChIP-seq, ATAC-seq, etc. Raw data will be published in NCBI's Gene Expression Omnibus per NCBI guidelines, while all processed datasets will be provided through the journal in which the work is published.

Sharing Model Organisms

All *C. elegans* strains generated in the completion of this proposal will be deposited into the C. elegans Genome Center (CGC), to which we have already deposited dozens of strains from previously published works (lab identifier AGD). All plasmids and expression vectors will be deposited to Addgene for sharing.

No custom antibodies or large-scale genomic data will be generated in the course of performing this project.

Authentication of Key Biological and/or Chemical Resources

We propose the following methods for the analyses of key biological and/or chemical resources described in this proposal:

Nematode Strains

All nematode strains synthesized in our lab, received from other labs, or ordered through the *Caenorhabditis Genetics Center* (CGC), will be validated using conventional single-worm genotyping to re-validate mutations and transgenes. All strains synthesized in our lab using mutagenesis, radiation-induced integration, or CRISPR-Cas9-mediated genome editing will be backcrossed to our N2 wild-type strain at least 8 times to eliminate background mutations and off-target effects. All nematode strains synthesized in this study will be deposited to the CGC.

Genetic drift is a major issue in *C. elegans*. We will take several precautions to prevent major consequences of genetic drift: 1) strains will be maintained at the ideal temperature of 15°C, with ample food, in darkness, and no starvation to prevent adaptations and evolutionary pressure, and 2) animals will only be maintained for a maximum of 3 months for mutations that cause a sick phenotype and 6 months for otherwise healthy animals, and will be re-thawed regularly from our -80°C backup.

Nematode Media

We have found that specific components of C. elegans media have an affect on the outcome of some experiments. Specifically, both the types of peptone and brands of agar used during experiment (vendor, composition) have the potential to affect physiological readouts in *C. elegans*. Moreover, age of plates and methods for IPTG induction all create variability in the efficacy of RNAi treatments. For all key experiments, we have tested effects across a variety of media components. Any results specific to a type of media will be carefully documented and reported clearly at the time of publication.

Antibodies

Key antibodies, including those against actin, fluorescent proteins, etc., will be validated by Western blot, immunoprecipitation, immunofluorescence, or flow cytometry (as described in the various Aims) using strains treated with RNAi to induce knockdown of the target protein or in the presence of competitive recombinant peptide against the epitope.

Chemicals

Purchased chemicals will be validated by analyses against published physiological phenotypes. No custom synthesized chemicals are proposed in this study.

Tissue-specific Promoters

Our experiments rely heavily upon the use of tissue-specific promoter markers to drive expression of proteins. Whenever possible, multiple tissue-specific promoters will be used and results will be cross-validated. For example, in testing tissue-specific neuronal expression, we routinely cross-compare between expression patterns seen with *unc-119*, *rab-3*, and *rgef-1*. Moreover, many of the promoters used in this study have already been heavily validated during our previous publication, and we have strong confidence in our promoters.