OMB Number: 4040-0010
Expiration Date: 12/31/2022

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
AG072902

b. Agency Routing Number

2. DATE SUBMITTED	**Application Identifier**	**c. Previous Grants.gov Tracking Number**
2020-12-08		GRANT13256110

5. APPLICANT INFORMATION **Organizational DUNS*:** 8007721620000

Legal Name*: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANTONIO
Department: Greehey Children's Cancer Res
Division: School of Medicine
Street1*: 7703 Floyd Curl Drive
Street2:
City*: San Antonio
County: Bexar
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 78229-3900

Person to be contacted on matters involving this application
Prefix: First Name*: Chris Middle Name: G. Last Name*: Green Suffix: CPA
Position/Title: Senior Director
Street1*: 7703 Floyd Curl Drive, MSC 7828
Street2:
City*: San Antonio
County: TX
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 782293900
Phone Number*: 210-567-2340 Fax Number: Email: grants@uthscsa.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-741586031-A3

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*
The dynamics and impact of R-loops in epigenetic stability and aging

12. PROPOSED PROJECT		**13. CONGRESSIONAL DISTRICTS OF APPLICANT**
Start Date*	Ending Date*	TX-021
08/01/2021	07/31/2024	

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Henry Middle Name: E Last Name*: Miller Suffix:
Position/Title: Graduate Research Assistant
Organization Name*: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANTONIO
Department: Cell Systems & Anatomy
Division: School of Medicine
Street1*: 8403 Floyd Curl Drive
Street2:
City*: San Antonio
County: Bexar
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 78229-3900
Phone Number*: 210-567-3716 Fax Number: Email*: millerh1@livemail.uthscsa.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$101,069.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$101,069.00
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*: Chris Middle Name: G. Last Name*: Green Suffix: CPA
Position/Title*: Senior Director
Organization Name*: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANTONIO
Department: Office of Sponsored Programs
Division:
Street1*: 7703 Floyd Curl Drive, MSC 7828
Street2:
City*: San Antonio
County: Bexar
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 78229-3900
Phone Number*: 210-567-2340 Fax Number: Email*: grants@uthscsa.edu

Signature of Authorized Representative*
Chris G. Green

Date Signed*
12/08/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:HM_F31_cover_letter1027949195.pdf

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

OMB Number: 4040-0010
Expiration Date: 12/31/2022

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:	UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANTONIO
Duns Number:	8007721620000
Street1*:	7703 Floyd Curl Drive
Street2:	
City*:	San Antonio
County:	Bexar
State*:	TX: Texas
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	78229-3900
Project/Performance Site Congressional District*:	TX-021

Additional Location(s) File Name:

Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T17:49:31.000-05:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

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*	HM_F31_project_summary1027949152.pdf
8. Project Narrative*	HM_F31_project_narrative1027949153.pdf
9. Bibliography & References Cited	HM_F31_bibliography1027949155.pdf
10.Facilities & Other Resources	HM_F31_facilities1027949205.pdf
11.Equipment	HM_F31_equipment1027949157.pdf

Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T17:49:31.000-05:00

PROJECT SUMMARY

Introduction: A growing body of evidence supports the notion that epigenetic dysregulation is a key driver of aging. Indeed, recent studies show that epigenetic markers accurately predict chronological age, and that *in vivo* epigenetic reprogramming can prolong lifespan and enable tissue regeneration in aged animals. Most striking was the recent evidence from the David Sinclair group which demonstrated that induction of epigenetic "noise" through use of non-mutagenic double-strand breaks (ICE mouse model) leads to accelerated aging phenotypes at the physiological and cellular level (ICE MEFs), such as loss of cell identity. Interestingly, the epigenetic aging induced in ICE mice results from the dysregulation of key enhancers, epigenomic structures which drive gene expression via 3D interactions with target gene promoters. Recent evidence indicates that enhancers are transcribed into a non-coding RNA species, enhancer RNA (eRNA), and that eRNA supports enhancer stability. Moreover, mounting evidence reveals that eRNA forms a structure with enhancer DNA called an "R-loop" (an RNA:DNA hybrid with a displaced ssDNA strand). In a recent study, our lab demonstrated that STAG2, a protein which helps maintain enhancer stability and cell identity, also binds R-loops *in vitro* and co-localizes with them at enhancers, suggesting that STAG2 binds eRNA R-loops. We also find (unpublished) that STAG2 protects R-loops from degradation by the RNA:DNA helicase RNaseH1. Furthermore, I found that DHX9, a protein which regulates R-loop formation, is the top over-expressed gene in ICE mouse muscle and I found evidence of eRNA R-loops at enhancers dysregulated in ICE MEFs. Taken together, these findings suggest that STAG2 protects eRNA R-loops to maintain youthful enhancer stability with age. Therefore, **I hypothesize that dysregulation of physiological R-loops drives epigenetic aging by impairing youthful enhancer stability**.

Aim 1: Elucidate the mechanism of eRNA R-loops in enhancer stability with epigenetic aging. I will uncover eRNA R-loops that are associated with enhancer dysregulation in epigenetic aging. I will use a CRISPR system to manipulate these R-loops and assess the impact on enhancer stability and cellular aging phenotypes.

Aim 2: Determine the impact of the STAG2/R-loop interaction in preserving the youthful epigenome. I will assess cellular aging and epigenetic noise in ICE cells with manipulation of STAG2 and RNaseH1, and I will assess the differential binding of STAG2 with epigenetic aging in ICE MEFs.

Conclusion and significance: With these aims, I will elucidate the role of physiological eRNA R-loops (and STAG2/R-loop interactions) in the mechanism of enhancer stability with aging. These aims are significant as they are the first to address the physiological role of R-loops in either enhancer stability or in aging, and they also have the potential to reveal novel drug targets for restoration of the youthful epigenome. Furthermore, the completion of the proposed training plan will prepare me for an independent research career in aging biology.

PROJECT NARRATIVE

Aging and age-related diseases are driven by epigenetic changes which may be reversible. There is strong evidence to suggest that the interaction of epigenomic elements called ‘enhancers’ and ‘R-loops’ may drive aging at the cellular level. By studying these interactions, we will expand our knowledge of epigenome aging and R-loops, while potentially revealing novel anti-aging therapeutic targets.

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FACILITIES & OTHER RESOURCES

Training Environment: UT Health San Antonio (UTHSA) is a leading academic research center which is rapidly expanding and driving the growth of the San Antonio Biomedical technology ecosystem. The Integrated Biomedical Sciences (IBMS) graduate program has over 300 faculty members with a range of research interests from basic science to clinical translation.

I am part of the Biology of Aging discipline, which provides training and research opportunities specific to my interests in aging research, an area of biomedical science which UTHSA has been a leader in for many years. It houses the Barshop Institute, a cornerstone of the international aging research ecosystem and a premier training site for aspiring aging researchers like myself. Biology of Aging discipline curricula are taught by Barshop faculty, covering aging biology at a molecular, cellular, and whole-physiology level as well as diseases of aging such as cancer and Alzheimer's. Furthermore, this discipline allows me to enhance my dry-lab skills by pursuing elective courses in biostatistics and data science. This combination of bioinformatics and aging coursework was instrumental to my ability to formulate and design the research aims in this proposal. With my long-term career goal of becoming an independent aging researcher, the Biology of Aging program at UTHSA is an ideal intellectual environment in which to receive training. My proposed research aims are largely centered around studying the aging epigenome. I have already enjoyed feedback from multiple Biology of Aging faculty about these aims and even refined my thinking on epigenetic aging by discussing it with some of them during my qualifying exam. This atmosphere of shared intellectual interest in aging will be advantageous as I complete my aims and, if successful, develop new conceptual models for the aging epigenome. Additionally, having a connection to so many aging researchers will be greatly beneficial for making the professional contacts necessary to find post-doc and, ultimately, faculty positions in the future. Furthermore, as part of the Biology of Aging program, I regularly attend Aging Biology Journal Club and Barshop seminars which both expose me to cutting-edge aging research topics from dynamic field leaders. Finally, the Barshop institute hosts an annual conference featuring talks from field leaders and at which I will give a yearly research presentation.

My sponsor, Dr. Bishop, in addition to being a Barshop Institute member, is also part of the Department of Cell Systems and Anatomy, a research-oriented interdisciplinary program with a wide range of expertise including cancer biology, cell and organelle biology, genetics and genomics, stem cell and developmental biology, and molecular biology of aging and age-related diseases. His lab is located in the Greehey Children's Cancer Research Institute (GCCRI), which provides an exciting, collaborative environment for biomedical research. GCCRI faculty perform research on a variety of topics, including DNA damage repair, RNA metabolism, computational biology, and pre-clinical drug testing. As a member of this institute, I have access to UTHSA and GCCRI core facilities including Genome Sequencing, Bioinformatics, Flow Cytometry, Optical Imaging, Macromolecular Structure, Histology and Immunohistochemistry, and Xenograft cores. Dr. Bishop is also a member of the Barshop Institute, which I previously described. Altogether, this creates a strong and collaborative research environment for the successful completion of my training plan and successful preparation for a career as an independent researcher in the biology of aging field. The GCCRI also provides regular skills and safety training relevant to laboratory techniques, such as cell culture.

Laboratory: The laboratory has all necessary equipment for molecular biology and tissue culture work. It contains 975 square feet of bench space and 225 square feet of tissue culture space, with six incubators and two biological safety hoods. These biological safety hoods prevent potential biohazards when working with cell lines, as is proposed herein. The laboratory also contains a combination refrigerator/freezer, a table-top, refrigerated centrifuge, two -80 freezers, and three -20 freezers. I also have access to GCCRI shared equipment rooms. The laboratory is equipped with a fume hood for use of hazardous chemicals.

Office: The GCCRI provides desk space for all students, postdocs, and faculty. An office of 128 square feet with additional adjacent file storage is available for Dr. Bishop. My desk space includes file storage space and a computer and access to shared office space with color printers, fax, and photocopiers/scanners. There are also two conference rooms equipped with projectors and video conferencing available for lab meetings, dissertation committee meetings, etc.

Computer: I have been provided with a Dell desktop computer with 64GB of RAM, 6 3.2GHz Intel i7-8700 processors, and a 1TB SSD. This workstation has all the tools I will need for my research, including R and Python software, Microsoft office, and Acrobat. The workstation also has a high-speed Ethernet connection to the school-wide network. I also have access to computers for equipment and microscopes as well as several high-performance computing Linux servers, including a 192-core Ubuntu 18.04 server with 500GB of RAM and a second 64-core Oracle Linux 7 server with 250GB of RAM. The GCCRI provides IT support, server facilities, and unlimited Box cloud storage services. Additionally, UTHSA has a license with Microsoft Azure which permits developing cloud-based web applications and databases. High-speed wireless internet access is available across the UTHSA campuses.

Other: The UTHSA library provides access to online databases and interlibrary loans. Additionally, Biology of Aging students can request assistance from the faculty discipline director and discipline coordinator in navigating class selection and other discipline-specific areas. In addition to the annual Barshop conference, the GCCRI also hosts events including annual research retreats and symposia.

EQUIPMENT

The Bishop Lab has all the equipment necessary for general cell and molecular biology. This includes gel electrophoresis for DNA and proteins, dot blot apparatus, six 96 well PCR machines, refrigerators, freezers, incubators, centrifuges as well as five microscopes, three dissecting and two inverted. One inverted microscope (Zeiss Axiovert 200 Mot) is fully automated capable of acquiring brightfield, DIC and fluorescent images in the X-Y-Z planes over time and in all formats from flasks to 384 well plates on a heated stage with either a Hamamatsu Orca ER or Q-Imaging color digital camera (2.120.02). This microscope is under the control of OpenLab software. In addition, we have Volocity deconvolution software for the manipulation of acquired image stacks. The other inverted fluorescent microscope is used for general tissue culture work. The three dissecting microscopes are used for mouse work, however, two are fully automated Zeiss Lumar, capable of acquiring brightfield and fluorescent images in the X-Y-Z planes over time and in all formats from flasks to 384 well plates using a cooled Zeiss CCD camera (2.120.02).

We have a multifunctional Molecular Devices M5 multiwell reader (luminometer, spectrophotometer and fluorometer) (3.200.R). This sits adjacent to a DW4 plate washer capable of dispensing/washing with 4 different liquids. These instruments are coordinated with a Synchromax robotic arm and stacking system as well as a barcode reader. All these instruments are synchronized under one computer program

Our tissue culture has six incubators, two are connected to N_2 supply allowing us to control O_2 levels by injecting N_2 gas as well as two biosafety hoods. Also housed in our tissue culture space is one Essen IncuCyte and a Seahorse 96XP as well as a hypoxia glove box. In addition, we have a 6 station BioTek Precision XS robot capable of multi channel, single channel and large volume dispensing into anything between test tubes to 384 well plates This is a fully programmable system. We also have a Matrix Wellmate for dispensing cells into 384 well plates. In addition, we also have an Amaxa nucleofection system with the 96-well shuttle component.

We have access to institutional equipment such as a scintillation counter (3.200.06E), a Typhoon Trio Phosphoimager (3.200.06C), a Jouan speedvac with a rotor for plates, 2 Beckman-Coulter Optima L-100 XP ultracentrifuges and 2 Avanti J-20 centrifuges, 1 Avanti J-20XPI with elutriation chamber, a microtome, an ABI 7500 Real-time PCR system(3.200.06C), an Agilent 2100 Bioanalyzer (3.200.06C)), a FACSCaliber analyst (3.200.06C), a HiSeq 3000 deep sequencer (2.120), an Illumina cBot for cluster generation (2.120), six Essen IncuCytes (within an incubator with oxygen suppression capability via nitrogen control; as noted, one of these is located in our own tissue culture suite), a BioFlux station, a Nikon spinning disc confocal microscope, and a Faxitron X-ray source accessible from both the the animal facility and the general lab space (1.194).

The Genome Sequencing Facility which is located within and supported by the GCCRI is equipped with Illumina HiSeq 3000, NextSeq500, and MiSeq sequencers, Illumina cBot Cluster Generation Station, Covaris S220 Ultra Sonicator, a 10x Genomics Chromium Controller, and Beckman Coulter SPRIworks Fragment Library System.

As a member of the Department of Cell Systems and Anatomy, we also have access to all large equipment available to the Department, including the Genomics Core and the Microscopy Core.

As a Programmatic member of Molecular Medicine (which is located across a small courtyard from the GCCRI) we have access to a laser scissor set-up. Also located in that building are our metabolomics and BASiC (Fluidigm and CyTOF single cell analyses) cores.

We also have access to biochemistry cores in an immediately adjascent building, particularly an NMR and X-ray crystalography core. Mass spec, SPR and ITC facilities are available on the main campus less than a mile away.

Within the Institute, we share a dark room (3.200.06B), two cold rooms (3.200.06D/3.200.02C), shared isotope laboratory (2.200.06C) and an autoclave (3.200.06A). As noted in the Facilities sheet, the GCCRI has invested in a significant amount of capital equipment that are freely available for our use. Further, we have established a RNAi high throughout screening core with an available Ambion RNAi library that we can access at discounted prices.

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Henry Middle Name E Last Name*: Miller Suffix:

Position/Title*: Graduate Research Assistant
Organization Name*: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANTONIO
Department: Cell Systems & Anatomy
Division: School of Medicine
Street1*: 8403 Floyd Curl Drive
Street2:
City*: San Antonio
County: Bexar
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 78229-3900

Phone Number*: 210-567-3716 Fax Number:

E-Mail*: millerh1@livemail.uthscsa.edu

Credential, e.g., agency login: MILLERH1

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

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: HM_F31_biosketch1027949163.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT13256238 Funding Opportunity Number: PA-21-051 . Received Date: 2020-12-08T17:49:31.000-05:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Alexander Middle Name James Last Name*: Bishop Suffix: PhD

Position/Title*: Associate Professor
Organization Name*: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANTONIO
Department: Cell Systems & Anatomy
Division: School of Medicine
Street1*: 8403 Floyd Curl Drive
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Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 78229-3900

Phone Number*: +1 210 562 9060 Fax Number: +1 210 562 9014

E-Mail*: BishopA@uthscsa.edu

Credential, e.g., agency login: bishopaj

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

Degree Type: PhD Degree Year: 1997

Attach Biographical Sketch*: File Name: AJRB_biosketch1027949161.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Yidong Middle Name Last Name*: Chen Suffix: PhD

Position/Title*: Professor
Organization Name*: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANTONIO
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Division: School of Medicine
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County: Bexar
State*: TX: Texas
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Country*: USA: UNITED STATES
Zip / Postal Code*: 78229-3900

Phone Number*: (210) 562-9163 Fax Number: (210) 562-9014

E-Mail*: Cheny8@uthscsa.edu

Credential, e.g., agency login: cheny8

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

Degree Type: PhD Degree Year: 1995

Attach Biographical Sketch*: File Name: YC_biosketch1027949162.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

NAME: Miller, Henry

eRA COMMONS USER NAME: millerh1

POSITION TITLE: Ph.D. candidate

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	START DATE	COMPLETION DATE	FIELD OF STUDY
Christopher Newport University, Newport News, Virginia	BS	08/2012	05/2016	Neuroscience (double with Philosophy)
The State University of New York at Buffalo, Buffalo, New York	MS	08/2016	05/2018	Biomedical Engineering
UT Health San Antonio, San Antonio, Texas	PhD	08/2018	08/2024	Biology of Aging

A. Personal Statement

My long-term goal is to become an independent researcher in the field of aging biology. Aging fascinated me ever since I was a child. Through my experiences in college and graduate school, I realized that this interest could become the basis for a rewarding research career. It is this independent research career in aging biology for which I am now training.

As a child, I was diagnosed with severe attention deficit disorder (ADD). Because of this, I received special education designed to help me understand my own brain. This led to a deep fascination with the mind. After high school, I attended college with the intention of earning a degree in psychology. However, freshman do not choose their classes, and I was enrolled in modern philosophy instead. Though it was not my first choice, the class fired my enthusiasm for formal logic and inspired me to pursue a philosophy major. Despite my strong freshman year grades, I was still struggling with my ADD. The medication I had taken throughout adolescence produced harsh side effects and, believing I could manage without, I discontinued it a few months before college. The light course-load and my excitement buoyed my initial academic performance. However, as sophomore year began, I found myself incapable of focusing. Furthermore, finding that philosophy did not hold my attention enough to support a long-term career, I searched for a second major to couple it with. My transcript reflects these circumstances in a long list of unrelated courses in which I often earned poor grades. In junior year, I thankfully found my passion in science, which motived me to resume ADD treatment and complete my neuroscience degree on time. Senior year, I also audited a math-based biochemistry course. I found this application of mathematics to the study of biological systems fascinating, and I subsequently applied for graduate-level education in biomedical engineering. As a master's student, I completed coursework that rapidly expanded my interests in drug development, biomedical commercialization, and computational biology, leading to my research projects in diagnostic biochips and nanomedicine as well as my experiences in two biotech startups. Moreover, I was finding that my long-time intellectual fascination in aging was quickly condensing into a driving research interest, eventually leading me to apply for PhD training at the renowned aging biology research institute at UT Health San Antonio.

In my first two years of PhD training, I have learned that aging is still a largely enigmatic process typically described in terms of 'hallmarks', the underlying causes of which are unclear. I also learned of the mounting evidence from recent studies which demonstrates that loss of epigenetic fidelity is a fundamental driver of aging at the cellular level. This showed me that the study of 'epigenetic aging' is an exciting new approach with the unprecedented potential to explain why we age and how to reverse diseases which largely result from aging, such as Alzheimer's Disease. However, the epigenome is a complex network of proteomic, metabolomic, and nucleic interactions which defies simple characterization. For this reason, I now aim to develop my capabilities in computational systems biology to model epigenetic aging and reveal novel anti-aging drug targets. Finding an advisor who shared these same goals, I excitedly joined his laboratory with co-mentorship from his computational collaborator. In this training, I will continue to develop my research skills both in the wet-lab and in computational biology, completing my dissertation research and preparing for an independent research career in aging biology.

B. Positions and Honors

Positions and Employment

2014	**Environmental Health Technician**, Fairfax County Health Department, Fairfax, VA
2014 - 2016	**Department Tutor**, Christopher Newport University, Newport News, VA
2016	**Biochemistry Course Tutor**, Christopher Newport University, Newport News, VA
2016	**Environmental Health Technician**, Fairfax County Health Department, Fairfax, VA
2016 - 2017	**Director of Operations**, Sonioptix LLC, Buffalo, NY
2017 - 2018	**Chief Communications Officer**, POP Biotechnologies, Buffalo, NY
2017 - 2018	**Lead Venture Coach**, Blackstone Launchpad at SUNY Buffalo, Buffalo, NY
2017 - 2018	**Project Manager (volunteer)**, School of Engineering, SUNY Buffalo, Buffalo, NY
2018 -	**Graduate Student Research Assistant**, UT Health San Antonio, San Antonio, TX

Other Experience and Professional Memberships

2017 - 2018	**Member**, Institute of Electrical and Electronics Engineers
2017 - 2018	**Member**, Biomedical Engineering Society
2017 - 2018	**Member**, Project Management Institute
2020 -	**Member**, International Society for Computational Biology
2020 -	**Graduate student representative**, Bioinformatics Curriculum Committee, UT Health San Antonio
2020 -	**Organizer and Primary instructor**, Bioinformatics Bootcamp Workshop, BIG Club, UT Health San Antonio, San Antonio, TX
2020 -	**Training Committee Chair**, BIG Club, UT Health San Antonio, San Antonio, TX

Academic and Professional Honors

2013	**Dean's List**, Christopher Newport University, Newport News, VA
2020	**Passed qualifying exam with honors**, UT Health San Antonio, San Antonio, TX
2020 - 2021	**Greehey Fellowship (pre-doctoral fellowship)**, UT Health San Antonio, San Antonio, TX

C. Contribution to Science

1. A novel nanomedicine for the treatment of peanut hypersensitivity (Lovell Laboratory, University at Buffalo, 2017-2018): Peanut hypersensitivity is a severe food allergy which affects 3-8% of all children. To date, no treatment has been approved which can prevent allergic reactions in these patients. Co-PoP is a porphyrin phospholipid which can enable liposomal delivery of antigens to the immune system and promote either immunogenicity or immune tolerance based on lipid composition. It was hypothesized that a formulation of Co-PoP liposomes could be created which would promote immune tolerance to peanut antigens as a novel treatment for peanut allergies. As a master's student researcher in the Jonathan Lovell group, I created the novel liposome formulation and demonstrated that the liposomes are stable within the expected size distribution. I then developed a mouse model of peanut hypersensitivity and began a pilot challenge study to test the drug in these mice. Preliminary evidence was observed that the drug produces immune tolerance in the mice, and these studies are still ongoing today. If successful, these studies will lead to the clinical translation of this therapeutic for use in human patients, with the potential to provide an effective treatment for peanut allergies.

2. CREB, Calcium, and Pathogenic Tau in Alzheimer's Disease (Frost Laboratory, UT Health San Antonio, 2018): Alzheimer's Disease (AD) is characterized at the cellular level by the pathogenic accumulation of tau and aberrant epigenetic changes such as cell cycle reactivation. Interestingly, it is also characterized by a dysregulation of intracellular calcium signaling, but the mechanism of this dysregulation and its consequences are unclear. It was subsequently hypothesized that tau accumulation causes a decrease in intracellular calcium signaling, leading to decreased CREB transcription factor binding and epigenetic dysregulation. While rotating in the Frost laboratory, I addressed this hypothesis by mining multiple sequencing studies to uncover CREB target genes downregulated with pathological tau accumulation. I also wrote the relevant methods and reviewed

the resulting manuscript prior to publication. The findings from this study not only provide support for the calcium hypothesis of AD, but also provide new insights into the cause of epigenetic dysregulation in AD neurons.

Mahoney R, Ochoa Thomas E, Ramirez P, **Miller HE**, Beckmann A, Zuniga G, Dobrowolski R, Frost B. Pathogenic Tau Causes a Toxic Depletion of Nuclear Calcium. **Cell Rep**. 2020 Jul 14;32(2):107900. doi: 10.1016/j.celrep.2020.107900. PMID: 32668249; PMCID: PMC7428851.

3. Computational techniques for studying complex epigenetic cell states (Bishop Laboratory, UT Health San Antonio, 2018-present): Traditional research approaches are not typically suitable for studying complex cellular interactions and epigenetic cell-state transitions, such as those found during stem cell differentiation. To elucidate these important biological interactions and transitions, such as those which underly epigenetic aging, it is necessary to develop computational models of these complex cell networks.

A. One area where this need arises is in the question of how cardiac non-myocytes differ with respect to sex and age in general and, particularly, in the context of cardiotoxic chemotherapy treatments (e.g., doxorubicin). It was hypothesized that neural networks would be effective for reconstructing complex biological relationships within the heart, particularly of cardiac non-myocytes. To address this hypothesis, I optimized a sparse autoencoder neural network on single cell RNA-Seq and mass spectrometry datasets. I also assisted in the design of key experiments and figures, and I reviewed the resulting manuscript. From these efforts, it was found that there are striking differences with respect to the proportion of endothelial cells in male and female mouse cardiac tissue, indicating an explanation for sex-specific differences in cardiac function with aging and chemotherapy. It was also found that neural networks effectively integrate datasets from different single cell modalities, a capability which will enable an unprecedented level of insight into the cell states of complex tissues by considering both protein expression and RNA transcription from millions of cells.

Iskra B, Davis L, **Miller HE**, Chiu Y, Bishop A, Chen Y, Aune G. Assessing the Heterogeneity of Cardiac Non-myocytes and the Effect of Cell Culture with Integrative Single Cell Analysis. [**Preprint**]. 2020 March 05. DOI: 10.1101/2020.03.04.975177.

B. A second area in which this need appears is in addressing the persistent challenge of determining from which cell of origin Ewing sarcoma (a pediatric bone cancer) arises. I hypothesized that I could reconstruct the cellular relationships of Ewing sarcoma to the normal tissues from which it might arise to reveal its cell of origin. I implemented a recently described manifold learning approach (PHATE) to reveal novel requirements for the transformation of normal cells into Ewing sarcoma tumors. Taken together, the findings suggested that Ewing sarcoma does not have one discrete cell of origin. Rather, a range of cell states are permissive for transformation as long as they fulfill certain key requirements. Furthermore, the study demonstrated that PHATE reveals cell states and cell state transitions with a resolution unmatched by other manifold learning approaches. With input from my advisors, I conceptualized and performed most of the experiments, designed most of the figures, and wrote the manuscript. I also presented this work as a lecture and as a poster.

Miller HE, Gorthi A, Bassani N, Lawrence LA, Iskra BS, Bishop AJR. Reconstruction of Ewing Sarcoma Developmental Context from Mass-Scale Transcriptomics Reveals Characteristics of EWSR1-FLI1 Permissibility. **Cancers (Basel)**. 2020 Apr 11;12(4):948. doi: 10.3390/cancers12040948. PMID: 32290418; PMCID: PMC7226175.

Miller, HE. "Reconstruction of Ewing Sarcoma Developmental Context from Mass-Scale Transcriptomics Reveals Characteristics of EWSR1-FLI1 Permissibility." Sarcoma Working Group Meeting. Greehey Children's Cancer Research Institute, 05, May, 2020, UT Health San Antonio, San Antonio. **Lecture**.

Miller HE, Gorthi A, Bassani N, Lawrence LA, Iskra BS, Bishop AJR. Reconstruction of Ewing Sarcoma Developmental Context in silico. **Poster** presented at: Cell Systems & Anatomy departmental retreat; 2020 May 18; San Antonio, Texas.

4. Dissecting the physiological roles of R-loops (Bishop Laboratory, UT Health San Antonio, 2018-present): R-loops are three-stranded structures formed by the hybridization of RNA and DNA. Physiological R-loops are formed as a natural biproduct of transcription, whereas pathological R-loops form as the result of transcriptional dysregulation, leading to genome instability. While the vast majority of R-loop research concerns their pathological role, mounting evidence suggests that physiological R-loops are vital for many biological

processes. Furthermore, R-loop mapping approaches are currently incapable of distinguishing different R-loop types. Consequently, many physiological R-loop roles remain uncharacterized.

A. In addressing one component of physiological R-loop activity, it was recently hypothesized that some R-loops serve as binding sites for cohesin complex members, in controlling 3D chromatin architecture. To address this hypothesis, I mined several public sequencing datasets to demonstrate the correlation between R-loop location and cohesin complex binding, generating the relevant figures, and writing the relevant methods. The resulting publication demonstrated that R-loops are readily bound by cohesin component STAG2, and, in particular, that STAG2 co-localizes with R-loops in multiple cell lines at enhancers, suggesting that R-loops may play a key physiological role in the epigenetic regulation of enhancer-promoter chromatin looping by STAG2.

Pan H, Jin M, Ghadiyaram A, Kaur P, **Miller HE**, Ta HM, Liu M, Fan Y, Mahn C, Gorthi A, You C, Piehler J, Riehn R, Bishop AJR, Tao YJ, Wang H. Cohesin SA1 and SA2 are RNA binding proteins that localize to RNA containing regions on DNA. **Nucleic Acids Res**. 2020 Jun 4;48(10):5639-5655. doi: 10.1093/nar/gkaa284. PMID: 32352519; PMCID: PMC7261166.

B. Another poorly understood aspect of R-loop physiology is their roles in controlling the dynamics of gene transcription. In an ongoing study in collaboration with the Ashok Venkitaraman laboratory at Cambridge University, it was hypothesized that BRCA2 degrades physiological R-loops which form during RNA polymerase pausing. To address this hypothesis, I analyzed multiple datasets from public and private sources and generated several key figures, demonstrating that BRCA2 prevents R-loop accumulation, and that this correlates with genome-wide changes in chromatin accessibility and gene expression. These preliminary results suggest a novel role for BRCA2 and R-loops in the control of gene expression.

C. Finally, to better understand the changes in R-loop physiological roles between biological conditions, it has been necessary to re-evaluate all previously published R-loop mapping data along with the approaches used to generate it. In an ongoing study in collaboration with the Fred Chedin laboratory at UC Davis, it was hypothesized that a standardized R-loop analysis pipeline, *RSeq*, could be developed and utilized to compile all available R-loop mapping data, allowing for the definition of R-loops which display evidence of physiological roles corresponding to different biological conditions and for benchmarks upon which to assess future R-loop mapping studies. To address this hypothesis, I developed the computational pipeline and have used it to re-analyze all public R-loop mapping datasets. This study will set standard practices for R-loop mapping analysis, reveal physiologically relevant R-loop subtypes, and enable hypothesis generation for future R-loop studies. The manuscript for this study, on which I will be first author, is currently in preparation.

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

Scholastic Performance

Year	Course Title	Grade	Year	Course Title	Grade
CHRISTOPHER NEWPORT UNIVERSITY			2016	Human Anatomy/Physiology II Lab	B-
2012	Public Speaking	A	2016	Organic Chemistry II	B-
2012	Introduction to Film Studies	B	2016	Organic Chemistry Lab II	B
2012	Indigenous Peoples in the 21st Century	B	2016	Jazz Combo (Piano)	A
2012	Jazz Ensemble (Piano & Sax)	A	2016	Research Methods in Neuroscience	B
2012	Modern Philosophy	A	2016	Neuroendocrinology	A
2012	Media and Crime	B-	2016	General Physics II *	A-
2013	Principles of Microeconomics	B+	2016	General Physics II Laboratory	A-
2013	Cabaret in the 20th Century	A-	2016	Cognitive Psychology	B+
2013	Jazz Ensemble (Piano & Sax)	A	**STATE UNIVERSITY OF NEW YORK AT BUFFALO**		
2013	Jazz Combo (Piano)	A	2016	Human Biology for Biomedical Engineering	A
2013	Music Technology	A	2016	Biomedical Micro/Nanotechnology	A-

2013	The Anatomy of Thought	A
2013	The Great Philosophers: Aquinas	A
2013	Principles of Macroeconomics	C+
2013	The Lotus Sutra	B-
2013	Ancient and Medieval Philosophy	C+
2013	The Great Philosophers: Plato	D
2014	British Pop Culture	C
2014	Civil War and Reconstruction	B
2014	Why People Believe Weird Things	B
2014	Introduction to Environmental Studies	C+
2014	Jazz Ensemble (Piano & Sax)	A-
2014	Jazz Combo (Piano)	A
2014	The Great Philosophers: Augustine	C
2014	Yoga: Philosophy and Practice	A
2014	Principles of Biology I	B
2014	Principles of Biology I Lab	B-
2014	General Chemistry I	B-
2014	General Chemistry Lab I	A
2014	Latin and Its Living Legacy	A-
2014	Critical Thinking I	A-
2014	Philosophy Senior Seminar	C+
2015	Principles of Biology III	B
2015	Neuroscience Capstone Project	B+
2015	Human Anatomy/Physiology I	B
2015	Human Anatomy/Physiology I Lab	C
2015	Organic Chemistry I	A-
2015	Organic Chemistry Lab I	B-
2015	Elementary Statistics	B
2015	Jazz Combo (Piano)	A
2015	Neurobiology	B
2015	Environmental Ethics	B
2016	Human Anatomy/Physiology II	C
2016	BioMEMS Fabrication Laboratory	A-
2016	Transport Phenomena 1 (Chemical Engineering Dept. course)	C+
2017	Tissue Engineering	A
2017	Biomaterials in Regenerative Medicine	A
2017	Biomechanics and Mechanobiology	A
2017	Medical Nanotechnology	A
2017	Individual Problems	A
2017	Masters Research	A
2018	Masters Research	A
	UT HEALTH SAN ANTONIO	
2018	Responsible Conduct of Research	S
2018	Lab Rotations	S
2018	Fundamentals of Biomedical Sciences	A
2019	Research	S
2019	Seminar	S
2019	Biology of Aging	A
2019	Experimental Design/Data Analysis	A
2019	Student Journal Club and Research Presentation	A
2019	Research	S
2019	Seminar	S
2019	Rigor and Reproducibility	A
2019	Scientific Writing	A
2020	Student Journal Club and Research Presentation	A
2020	Qualifying Exam	H
2020	Research	H
2020	Seminar	S
2020	Genomic Data Analysis	A

S - satisfactory, H – honors, * course completed via challenge

Christopher Newport University: **3.07 GPA**
The State University of New York at Buffalo: **3.788 GPA**
University of Texas Health Science Center at San Antonio: **4.0 GPA**

GRE scores (Fall 2014):
- Verbal Reasoning: 163, **93rd percentile**
- Quantitative Reasoning: 160, **76th percentile**
- Analytical Writing: 5.0, **93rd percentile**

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

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: Alexander J. Bishop

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

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Leicester University, UK	B.Sc. (Hon)	06/1993	Biological Sciences
Institute of Molecular Medicine, Oxford University, UK	D.Phil.	05/1998	Molecular Genetics
Harvard School of Public Health, Boston, MA, USA	Postdoc	02/2001	Cancer Cell Biology
Harvard School of Medicine, Boston, MA, USA	Postdoc	05/2005	Genetics

A. Personal Statement

My research focus is on DNA damage response and DNA repair, with a particular interest in Ewing sarcoma, breast cancer and Ataxia telangiectasia. As a basic scientist my lab uses a variety of methods; genetics, molecular biology, cell biology and mouse models (genetic and tumor models). Of my 50+ peer reviewed publications, half are on DNA repair, particularly homologous recombination. Over the last 20 years I expanded my research program to use genomic level approaches to facilitate my research interests, from microarray analyses, to genome wide RNAi screening and then many genomic sequencing technologies. We have also developed significant expertise in R-loops biology and how it relates to DNA repair and replication. Importantly we established key techniques to evaluate R-loops, including DRIPseq and RNAPII ChIPseq. The relationship between these various endeavors and expertise is most apparent in the work we published about two years ago (*Nature* 2018) outlining the transcriptional dysregulation that occurs in Ewing sarcoma, another recently published paper where we contributed the R-loop analyses for ETMR (*Nature*, 2019). Another collaborative project examining R-loops in rDNA and how they function to maintain phase separation of nucleolar bodies (and how they are disrupted in Ewing sarcoma) was just published in *Nature* (2020). With this background we have become interested in understanding the normal physiological roles of R-loops, impact on gene expression programs and pathological consequences associated with their dysregulation, which is very different from how many others look for pathological conflict between R-loops and replication to impact genome stability (which we also examine, but more as a potential therapeutic strategy to treat cancer).
Since becoming an independent investigator, I have trained five postdoctoral fellows all of whom remained in science, four of whom are continuing their academic careers, one is now tenured, one is tenure track and one is currently a non-tenure track Assistant Professor. One postdoc is pursuing additional training while, yet another has joined a biotech company. Two additional postdoctoral fellows are currently under training. I have taken a special interest in advancing postdoctoral fellows through their careers. In fact, since coming to UTH-SA, I have developed a postdoctoral career workshop, am a member of a Departmental committee to promote postdoctoral career advancement and most recently initiated a Departmental Postdoctoral Seminar Series. I am therefore a strong advocate for finding means for postdoctoral fellows to achieve their career goals. I have also been fortunate enough to have recruited several talented graduate students, six have now graduated and all pursued further research careers in either academia or private industry. I am currently training four graduate students. Over the last few years five of my graduate students obtained independent funding (including DoD BRCP program and PCRCP Horizon Award), as did three of my postdocs (including DoD BCRP and AstraZeneca-AACR START). I am therefore wholly committed to training young scientists such as Henry and seeing them succeed in their career.

B. Positions and Honors

Graduate Student (Borts):	Institute of Molecular Medicine, Oxford University, UK	1993 - 1998
Postdoctoral Fellow (Schiestl):	Department of Cancer Cell Biology, HSPH, Harvard, MA	1997 - 2001
Postdoctoral Fellow (Leder):	Department of Genetics, Harvard Medical School, MA	2001 - 2005
Assistant Professor:	Cellular and Structural Biology, UT Health San Antonio, TX	2005 - 2013
Principal Investigator	Greehey Children's Cancer Research Institute	2005 - present
Associate Professor:	Cell Systems and Anatomy, UT Health San Antonio, TX	2013 - present

Other Experience and Professional Memberships

Member	Mays Cancer Center	2005 - present
Member	Nathan Shock Aging Center and Barshop Institute	2005 - present
Member	American Society for Microbiology	2000 - present
Member	Environmental Mutagenesis Society	2010 - present
Member	American Association for Cancer Research	2010 - present
Associate Member	Children's Oncology Group (COG)	2011 - present
Member	Ewing Sarcoma Biology SubCommittee for COG	2011 - present
Academic Editor	PLoS ONE	2011 - 2016
Committee member	California Breast Cancer Research Program	2012 - 2014
Ad hoc committee member	DoD CDMRP BCRP, PCRP and RCRP	2015 - present
Ad hoc committee member	NIH NCI CE, MCT1	2015 - present
Ad hoc committee member	Dept of Veterans Affairs ONCC	2015 - present
Member	UTHSCSA's Academy of Master Teachers	2015
Member	UT Shine Academy	2017 - present
Member planning committee	AACR San Antonio Breast Cancer Symposium	2018 - present

Honors

MRC (UK) Graduate Student Fellowship		1993 - 1997
ACS Postdoctoral Fellowship (not accepted due to F32 Fellowship award)		1997
F32 GM19147, NIGMS Postdoctoral Fellowship,		1997 - 2000
Keystone Scholarship	"Molecular Basis of Cancer", NM	1999
ASM Travel Grant	"DNA Repair and Mutagenesis", SC	1999
Keystone Scholarship	"Cell Cycle/Molecular Basis of Cancer", NM	2001
Keystone Scholarship	"Replication and Recombination", UT	2002
K22 ES-02-006, NIEHS Transition to Independent Positions Award,		2004 - 2010
NCI Travel Award	"Systems Level Understanding of DNA Damage Responses", Netherlands	2009
Voelcker Fund Young Investigator Award		2010
CSA Award for Excellence in Graduate Student Education		2010
AACR Minority-Serving Institution Faculty Scholar in Cancer Research Award		2013
UTHSCSA 2015 Presidential Teaching Excellence Award		2015
ATW2015 Travel Award to attend the 2015 Ataxia Telangiectasia Workshop in China		2015
AACR Minority-Serving Institution Faculty Scholar in Cancer Research Award		2015
AACR Minority-Serving Institution Faculty Scholar in Cancer Research Award		2017
Mays Cancer Center, Discovery of the Year		2018

C. Contributions to Science

1. R-loops and Ewing sarcoma: The major focus of the lab in recent years has been in investigating why Ewing sarcoma is sensitive to a wide spectrum of DNA damaging agents and how they develop resistance. Our investigations into the biology of Ewing sarcoma led us to identifying a dysregulation in control of transcription and the accumulation of R-loops. Having established many of the techniques necessary to evaluate R-loops we are now extending these studies to better understand their occurrence in normal and diseased tissues, mainly focused on Ewing sarcoma but also applying to other diseases.

1) Abraham KJ, Chan JNY, Nein Khosraviani N, Gorthi A, Samman A, Zhao DY, Wang M, Singhania R, Ostrowski LA, Oshidari R, Pietrobon V, Ohh M, Dickson BC, De Carvalho DD, Lee S, Greenblatt JF, **Bishop AJR**, Mekhail K. Nucleolar RNA polymerase II drives ribosome biogenesis. Nature 2020 Sep; 585(7824):298-302 PMID: 32669707; PMCID: PMC7486236

2) Miller HE, Gorthi A, Bassani N, Lawrence LA, Iskra BS and **Bishop AJR**, Reconstruction of Ewing Sarcoma Developmental Context from Mass-Scale Transcriptomics Reveals Characteristics of EWSR1-FLI1 Permissibility. Cancers 2020 Apr 11; 12(4):948
3) Schafer ES, Rau RE, Berg S, Liu X, Minard CG, **Bishop AJR**, Romero JC, Hicks MJ, Nelson, Jr, MD, Voss S, Reid JM, Fox E, Weigel BJ and Blaney SM. Phase 1/2 trial of talazoparib in combination with temozolomide in children and adolescents with refractory/recurrent solid tumors including Ewing sarcoma: a Children's Oncology Group Phase 1 Consortium study (ADVL1411). Pediatric Blood & Cancer 2019 Nov 14:e28073
4) Gorthi A, Romero JC, Loranc E, Cao L, Lawrence LA, Goodale E, Balboni-Iniguez A, Bernard X, Masamsetti VP, Roston S, Lawlor ER, Toretsky JA, Stegmaier K, Lessnick SL, Chen Y and **Bishop AJR**. EWS-FLI1 Increases Transcription to cause R-Loops and Block BRCA1 Repair in Ewing Sarcoma. Nature 2018 Mar 15;555(7696):387-391

2. Work on DNA repair: The majority of my early work involved the investigation between various genetic backgrounds and homologous recombination in a variety of contexts, mainly using mouse models (knockouts, conditional systems, transgenic models, etc). This work built from my training on homologous recombination in yeast during my PhD and extended from my postdoctoral training into my own independent group. Gene knockouts (constitutive and conditional) we have published on include ATM, ATR, p53, p21, GADD45a, BRCA1, Ku80, WRN, BLM and PARP1 as well as various drug exposures (benzo(a)pyrene, X-rays, cisplatin, MMS, EMS, etoposide, camptothecin, PARP1 inhibitor and hydroxyurea), sometimes in the context of these genetic backgrounds; publications were in journals such as Cancer research, NAR, MCB, DNA repair, etc.

1) Karia B, Martinez JA, **Bishop AJR.** Induction of Homologous Recombination Following *in utero* Exposure to DNA-Damaging Agents. DNA Repair 2013 Nov; 12(11):912-921
2) Brown AD, Claybon AB, **Bishop AJR**. A conditional mouse model for measuring the frequency of homologous recombination events *in vivo* in the absence of essential genes. Molecular and Cellular Biology 2011 Sep; 31(17):3593-602
3) Claybon AB, Karia B, Bruce C and **Bishop AJR**. PARP1 suppresses homologous recombination events in mice in vivo Nucleic Acids Research 2010 Jul; 38: 7538-7545
4) **Bishop AJR**, Barlow C, Wynshaw-Boris AJ and Schiestl RH. Atm deficiency causes an increased frequency of intrachromosomal homologous recombination in mice. Cancer Res 2000 Jan; 60(2):395-399.

3. Damage response: In addition to the contributions described above, I have also been investigating mechanisms of damage survival using comparative biology, RNAi screening, gene expression, metabolomics and bioinformatics approaches in tissue culture systems. For this we have had to develop our own bioinformatics and statistical approaches but now work closely with a team of bioinformaticians and computer scientists. In general, we investigate mechanisms of damage survival, the damages usually being chemotherapeutics, applying this knowledge to the treatment of cancer. Most recently we have developed a significant interest into understanding the biology of NRF2 and how this protein relates to damage response, metabolic control and mechanisms of survival including unfolded protein response and ferroptosis.

1) Zanotto-Filho A, Rajamanickam S, Loranc E, Masamsetti P, Gorthi A, Romero JC, Tonapi SS, Goncalves RM, Reddick RL, Benavides R, Kuhn J, Chen Y and **Bishop AJR**. Sorafenib improves alkylating therapy by blocking induced inflammation, invasion and angiogenesis in breast cancer cells. Cancer Letters 2018 Jul; 425: 101-115
2) Zanotto-Filho A, Masamsetti P, Loranc E, Tonapi SS, Gorthi A, Bernard X, Goncalves RM, Moreira JCF, Chen Y and **Bishop AJR**. Alkylation-induced NRF2 blocks endoplasmic reticulum stress-mediated apoptosis via control of glutathione pools and protein thiol homeostasis. Molecular Cancer Therapeutics 2016 Dec; 15(12):3000-3014
3) Zanotto-Filho A, Dashnamoorthy R, Loranc E, de Souza LHT, Moreira JCF, Suresh U, Chen Y, **Bishop AJR**. Combined gene expression and RNAi screening to identify alkylation damage survival pathways from fly to human PLoS One 2016 Apr; 11(4): e0153970
4) Ravi D, Wiles AM, Bhavani S, Ruan J, Leder P and **Bishop AJR**. A network of conserved damage survival pathways revealed by a genomic RNAi screen. PLoS Genetics 2009 Jun: 100052-100052.

4. Collaborative work: I have participated in several studies attempting to understand how cells recognize and respond to different forms of damage, particularly in the context of a genetic deficiency in some component of DNA damage response or DNA repair and how this can be applied to cancer.

1) Lambo S, Gröbner S, Rausch T, Waszak S, Schmidt C, Gorthi A, Romero JC, Mauermann M, Brabetz S, Krausert S, Buchhalter I, Koster J, Sill M, Hübner J, Mack N, Schwalm B, Ryzhova M, Hovestad V, Papillon-Cavanagh S, Chan J, Landgraf P, Ho B, Milde T, Witt O, Ecker J, Sahm F, Sumerauer D, Ellison D, Orr B, Darabi A, Haberler C, Figarella-Branger D, Wesseling P, Schittenhelm J, Taylor M, Gil-da-Costa M, Łastowska M, Grajkowska W, Hasselblatt M, Hauser P, Pietsch T, Uro-Coste E, Bourdeaut F, Masliah-Planchon J, Rigau V, Li XN, Schüller U, Snuderl M, Karajannis M, Giangaspero F, Jabado N, von Deimling A, Jones D, Korbel J, von Hoff K, Lichter P, Huang A, **Bishop A**, Pfister S, Korshunov A, Kool M. The Molecular Landscape of Embryonal Tumors with Multilayered Rosettes at Diagnosis and Relapse. Nature 2019 Dec 12; 576(7786):274-280
2) Countryman P, Fan Y, Gorthi A, Pan H, Strickland J, Kaur P, Wang X, Lin J, Lei X, White C, You C, Wirth N, Tessmer I, Piehler J, Riehn R, **Bishop AJR**, Tao YJ, Wang H. Cohesin SA2 is a sequence-independent DNA-binding protein that recognizes DNA replication and repair intermediates. J Biol Chem 2018 Jan;293(3):1054-1069
3) Rajamaniskcam S, Panneerdoss S, Gorthi A, Timilsina S, Kovalsky D, Hanes M, Vadlamudi R, Chen Y, **Bishop AJ**, Arbiser JL and Rao MK. FOXM1-Mediated DNA repair by Imipramine Blue Suppresses Breast Cancer Growth and Metastasis Clinical Cancer Research 2016 Feb;
4) Lee IH, Cao L, Kawai Y, Fergusson MM, Liu J, Rovira I, **Bishop AJR**, Motoyama N, Komatsu M and Finkel T. Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress. Science 2012 Apr; 336(6078): 225-228

A Complete List of Published Work in MyBibliography includes 58 publications:
http://www.ncbi.nlm.nih.gov/sites/myncbi/alexander.bishop.1/bibliograpahy/40269339/public/?sort=date&direction=ascending

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

Ongoing Research Support (no overlap with the current proposal)

SU2C-CRUK Pediatric Cancer New Discoveries Challenge (*PI: Bishop*) 12/2020 - 11/2022
Targeting R-loop stability in Ewing sarcoma
To determine the therapeutic utility of targeting R-loop stabilizing mechanisms in Ewing sarcoma.

NIH/NCI R01 CA241554 (*PI: Bishop*) 05/2020 - 04/2025
Dysregulated transcription processes in Ewing sarcoma
To determine the consequences of dysregulated transcription regulation in Ewing sarcoma.

CPRIT RP170345 (*PI: Oyajobi, Role: CoI*) 12/01/16 - 11/20/21
UTHSCSA Cancer Research Training Program
Research Training Award supports for pre-doctoral, post-doctoral trainees, and summer (undergraduate) students, as part of a comprehensive training program covering all aspects cancer research.

The Andrew McDonough B+ Foundation #614252: Research Grant (*PI: Bishop*) 01/2019 - 12/2020
Targeting RNA processing defects of Ewing sarcoma
To follow up on initial validations of a previously conducted RNAi screen that identified splicing components as synthetic lethal target in Ewing sarcoma. The aim is to then evaluate splicing inhibitors that have the same effect as RNAi depletion of splicing genes and if a therapeutic window exists that can that suggests these inhibitors can be used in the treatment of Ewing sarcoma.

IIMS/GCCRI Pilot funds (*PI: Bishop*) (NCE to 04/2020) 05/2017 - 04/2019
Targeting the transcription dysregulation of Ewing sarcoma
To validate an RNAi screen for synthetic lethal viability in Ewing sarcoma to identify determinants of transcription regulation and therapeutic targets that can be used in the treatment of Ewing sarcoma.

GCCRI pilot experiment support (*PI: Bishop*) 09/2019 - 08/2020
Metabolomics in ATM inhibited cells
Isotope tracing metabolomics experiment in cells +/- ATM inhibitor for glucose and glutamine utilization.

MERCK EMD Serano (*PI: Bishop*) 10/2019 – 10/2021
Assessing the accumulation of R-loops in cancer as an indication of sensitivity to RNA splicing inhibition
To identify whether high R-loops indicate splicing defects in cancers and sensitivity to splicing inhibitors.

Completed Research Support

CPRIT RP150445: IIRACCA (*PI: Bishop*) (NCE to 08/2019) 03/2015 - 02/2019

Ewing's sarcoma, a homologous recombination defective disease

Najim Foundation and GCCRI funds Award (*PI: Bishop*) 07/2015 - 06/2016
To conduct a siRNA viability screen in Ewing sarcoma cells to identify new targets for treatment.

Hyundai Hope on Wheels Hope Grant Award (*PI: Bishop*) 09/2012 - 08/2014
Homologous recombination defect in malignant myeloid diseases in children

San Antonio Area Foundation 152711 Biomedical Research Grant (*PI: Bishop*) 05/2012 - 04/2013
Targeting altered nucleotide pool to treat cancer cells

NIH NCI 1R01CA179120 (*PI: Rao, Role: co-I*) 02/2015 - 01/2020
miRNAs: Safe and effective therapeutic adjuvants for treating drug resistant TNBC

NIH/NCI R01CA152063 (*PI: Bishop*) (NCE to 07/2018) 09/2011 - 07/2016
Improving etoposide treatment of Ewing's sarcoma

Helen Freeborn Kerr Charitable Foundation Award (*PI: Bishop*) 09/2011 - 04/2012
Identifying mechanisms involved in cancer development and novel means to prevent cancer forming

Max and Minnie Tomerlin Voelcker Fund Young Investigator Award (*PI: Bishop*) 07/2010 - 06/2013
Determining cisplatin survival factors to augment ovarian cancer treatment

ACS RSG-10-049-01-DMC Research Grant (*PI: McEwan, CoI: Bishop*) 01/2010 - 12/2013
Integration of the Jun N-terminal Kinase and p53 signaling pathways

NIH/NIEHS R15ES019128 (*PI: Bishop)* 05/2010 - 10/2013
Comparing and contrasting the biology of damage survival at a genomic level

ACS 122295 Pilot (*PI: Bishop)* 08/2005 - 08/2006
Using conditional mutants to investigate recombination

NIH/NIEHS K22 ES-02-006 (*PI: Bishop*) (NCE to 05/2010) 09/2004 – 09/2007
A screen for damage response using recombination

NIH/NIGMS F32 GM19147 Postdoctoral Fellowship (*PI: Bishop*) 07/1997 - 06/2000
Involvement of p53 and Atm in homologous recombination

Medical Research Council UK Predoctoral Fellowship (*PI: Bishop*) 09/1993 - 08/1996
The dynamics of minisatellite changes during meiosis in the yeast Saccharomyces cerevisiae

Ongoing Fellowships to trainees

Greehey Graduate Predoctoral Fellowship *(PI: Miller, Role: Mentor)* 09/2020 - 08/2021
Evaluating R-loops with new bioinformatics tools.

Mays Cancer Center Fellowship Predoctoral Fellowship (*PI: Kanda,* 08/2020 - 07/2022
Role: Mentor). *Ewing Sarcoma relies on Endogenous Cysteine and Glutamine for Antioxidant Response.*

CPRIT Training Grant RP170345 Predoctoral Fellowship (*PI: Mukhopadhyay,* 10/2020 - 09/2021
Role: Mentor). *Evaluate replication stress in Ewing sarcoma.*

AACR-AstraZeneca START fellowship (*PI: Gorthi, Role: Mentor*) 08/2018 - 07/2021
Identifying Modifiers of PARP1 Inhibitor Sensitivity in BRCA-like Tumors

CDMRP PRCRP Horizon Fellowship CA181177 (*PI: Lawrence, Role: Mentor*) 08/2019 - 07/2021
Transcription, R-Loops, and RNA Splicing in Ewing Sarcoma

Completed Fellowships to trainees

NCATS TL1TR002647 Postdoctoral fellowship *(PI: Gorthi, Role: Co-Mentor)* 07/2018 - 06/2019
NCATS TL1TR002647 Predoctoral Training Fellowship (*PI: Lawrence, Role: Mentor*) 07/2018 - 06/2020
NIH/NCI T32CA148724 Postdoctoral fellowship *(PI: Gorthi, Role: Co-Mentor)* 09/2015 - 08/2017
Greehey Graduate Predoctoral Fellowship *(PI: Sybouts, Role: Mentor)* 09/2015 - 08/2016
CPRIT Training Grant Postdoctoral Fellowship *(PI: Romero, Role: Mentor)* 09/2015 - 08/2016
DoD CDMRP BCRP Postdoctoral Fellowship *(PI: Zanotto-Filho, Role:Mentor)* 12/2013 - 11/2016
Greehey Graduate Predoctoral Fellowship *(PI: Chavez-Santosco, Role: Mentor)* 09/2013 - 08/2014
NIH/NCI T32CA148724 Predoctoral Fellowship (*PI: Tonapi, Role: Mentor*) 07/2013 - 06/2015
CPRIT Training Grant Predoctoral Fellowship (*PI: Gorthi, Role: Mentor*) 10/2012 - 09/2013
Greehey Graduate Predoctoral Fellowship *(PI: Gorthi, Role: Mentor)* 09/2011 - 08/2012
DoD CDMRP BCRP BC093931 Predoctoral Fellowship (*PI: Karia, Role: Mentor*) 01/2010 - 12/2012
NIH/NIA 5T32AG021890 Predoctoral Fellowship (*PI: Brown, Role: Mentor*) 05/2007 - 04/2012

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02-28-2023)

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: Chen, Yidong

eRA COMMONS USER NAME (credential, e.g., agency login): cheny8

POSITION TITLE: Professor, Dept. of Population Health Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University, Shanghai	BS	06/1983	Electrical Engineering
Fudan University, Shanghai	MS	06/1986	Electrical Engineering
Rochester Institute of Technology, Rochester, New York	PHD	05/1995	Imaging Science

A. Personal Statement

For this F31 predoctoral fellowship, I will be glad to participate as a co-mentor for the Henry Miller, especially in the area of computational biology. In the past, I have mentored several graduate students and post-doctoral fellows, two of them are successfully acquired research support through AACR post-doctoral fellowship and NCI-K99/R00. My research focuses on bioinformatics, computational modeling, and biostatistics for the application of gene expression, miRNA, DNA copy number, SNP and other genome-wide profiling for cancer research, especially for pediatric cancer studies. Currently, I am the director of the computational biology and bioinformatics program at Greehey Children's Cancer Research Institute (GCCRI), and Professor in the Department of Population Health Sciences (PHS, School of Medicine) at the UTHSA. Prior to joining the PHS, I was a staff scientist at National Cancer Institute/NIH specializing in Bioinformatics, and Associate Investigator at National Human Genome Research Institute/NIH (1996-2006), leading the bioinformatics efforts for microarray technology. I authored and co-authored about 200 peer-reviewed papers in the areas of prostate cancer, breast cancer, melanoma in journals such as *Journal of Bioinformatics, Nature, NEJM, PNAS, JNCI* and *Cancer Research*. I currently lead the bioinformatics effort in next-generation sequencing, as the Principal Investigator funded by Cancer Prevention and Research Institute of Texas (CPRIT). In addition, my lab is continuing efforts on developing new data analysis methods for next-generation sequencing, microarray, and other high-throughput technologies, and is providing statistical analysis tools for research results of integrative data analysis, utilizing NCBI GEO data or TCGA data for comparative studies. I have the expertise, leadership, and motivation necessary to carry out the mentoring duty required in the proposal. As a leader in the field of bioinformatics, I have, along with my colleagues at various institutes, mentored students and fellows (see various publications), successfully organized international/national bioinformatics conferences, and participate in various teaching and educational programs to promote the technologies and bioinformatics methodologies to students from biological and clinical sciences, as well as computational sciences.

1. Chiu YC, Chen HH, Gorthi A, Mostavi M, Zheng S, Huang Y, **Chen Y**. Deep learning of pharmacogenomics resources: moving towards precision oncology. Brief Bioinform. 2019 Dec 8;PubMed PMID: 31813953
2. Gorthi A, Romero JC, Loranc E, Cao L, Lawrence LA, Goodale E, Iniguez AB, Bernard X, Masamsetti VP, Roston S, Lawlor ER, Toretsky JA, Stegmaier K, Lessnick SL, **Chen Y**, Bishop AJR. EWS-FLI1 increases transcription to cause R-loops and block BRCA1 repair in Ewing sarcoma. Nature. 2018 Mar 15;555(7696):387-391. PubMed PMID: 29513652.
3. Panneerdoss S, Eedunuri VK, Yadav P, Timilsina S, Rajamanickam S, Viswanadhapalli S, Abdelfattah N, Onyeagucha BC, Cui X, Lai Z, Mohammad TA, Gupta YK, Huang TH, Huang Y, **Chen Y**, Rao MK. Cross-talk among writers, readers, and erasers of m6A regulates cancer growth and progression. Sci Adv. 2018 Oct;4(10). PubMed PMID: 30306128; PubMed Central PMCID: PMC6170038. (co-corresponding authors).
4. Panneerdoss S, Viswanadhapalli S, Abdelfattah N, Onyeagucha BC, Timilsina S, Mohammad TA, **Chen Y**, Drake M, Vuori K, Kumar TR, Rao MK. Cross-talk between miR-471-5p and autophagy component proteins

regulates LC3-associated phagocytosis (LAP) of apoptotic germ cells. Nat Commun. 2017 Sep 19;8(1):598. PubMed PMID: 28928467; PubMed Central PMCID: PMC5605700.

B. Positions and Honors

Positions and Employment

1986 - 1988	Assistant Professor, Fudan University, Shanghai
1988 - 1989	Visiting scholar, Dept. of Computer Engineering, Rochester Institute of Technology, Rochester, NY
1995 - 1996	Research Engineer, Hewlett-Packard Company, Vancouver, WA
1996 - 2001	Special Expert, Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD
2002 - 2006	Associate Investigator, Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD
2006 - 2008	Staff Scientist, Genetics Branch, NCI/NIH, Bethesda, MD
2008 - 2009	Assistant Professor, Dept. of Epidemiology and Biostatistics, UTHSA, San Antonio, TX
2009 - 2019	Professor, Dept. of Epidemiology and Biostatistics, UTHSA, San Antonio, TX
2014 - 2019	Co-Director, Next-generation sequencing shared Resource, Cancer Therapy and Research Center, UTHSA, San Antonio, TX
2008 -	Director, Computational Biology and Bioinformatics, Greehey Children's Cancer Research Institute, San Antonio, TX
2019 -	Professor, Dept. of Population Health Sciences, UTHSA, San Antonio, TX
2011 -	Faculty Advisor, Genome Sequencing Facility, UTHSA, San Antonio, TX
2014 -	Director, Biostatistics and Bioinformatics Shared Resource, Mays Cancer Center, UTHSA, San Antonio, TX

C. Contribution to Science

1. My earlier research work mainly focused on microarray data analysis, including the **pioneering ratio statistics for gene expression measurement, multidimensional scaling for tumor sample clustering and classification** since 1997. Since then, we have applied these methods in many cancer studies, such as melanoma, breast cancer, and hepatocellular carcinoma.
   a. **Chen Y**, Dougherty ER, Bittner ML. Ratio-based decisions and the quantitative analysis of cDNA microarray images. J Biomed Opt. 1997 Oct;2(4):364-74. PubMed PMID: 23014960.
   b. **Chen Y**, Kamat V, Dougherty ER, Bittner ML, Meltzer PS, Trent JM. Ratio statistics of gene expression levels and applications to microarray data analysis. Bioinformatics. 2002 Sep;18(9):1207-15. PubMed PMID: 12217912.
   c. **Chen Y**, Meltzer PS. Gene expression analysis via multidimensional scaling. Curr Protoc Bioinformatics. 2005 Jul; Chapter 7:Unit 7.11. PubMed PMID: 18428752.
   d. Xiao Y, Hsiao TH, Suresh U, Chen HI, Wu X, Wolf SE, **Chen Y**. A novel significance score for gene selection and ranking. Bioinformatics. 2014 Mar 15;30(6):801-7. PubMed PMID: 22321699; PubMed Central PMCID: PMC3957066.

2. We have recently **developed and implemented a set of analysis tools for next generation sequencing tasks** to support our Genome Sequencing Facility. Particularly, tools for RNA-seq, mutation analysis, MBDCap-seq and ChIP-seq has been employed in many cancer studies and result in many peer-reviewed publications.
   a. Gu F, Doderer MS, Huang YW, Roa JC, Goodfellow PJ, Kizer EL, Huang TH, **Chen Y**. CMS: a web-based system for visualization and analysis of genome-wide methylation data of human cancers. PLoS One. 2013;8(4):e60980. PubMed PMID: 23630576; PubMed Central PMCID: PMC3632540.
   b. Zhao W, Li Q, Ayers S, Gu Y, Shi Z, Zhu Q, **Chen Y**, Wang HY, Wang RF. Jmjd3 inhibits reprogramming by upregulating expression of INK4a/Arf and targeting PHF20 for ubiquitination. Cell. 2013 Feb 28;152(5):1037-50. PubMed PMID: 23452852; PubMed Central PMCID: PMC3742052.
   c. Dave B, Granados-Principal S, Zhu R, Benz S, Rabizadeh S, Soon-Shiong P, Yu KD, Shao Z, Li X, Gilcrease M, Lai Z, **Chen Y**, Huang TH, Shen H, Liu X, Ferrari M, Zhan M, Wong ST, Kumaraswami M,

Mittal V, Chen X, Gross SS, Chang JC. Targeting RPL39 and MLF2 reduces tumor initiation and metastasis in breast cancer by inhibiting nitric oxide synthase signaling. Proc Natl Acad Sci U S A. 2014 Jun 17;111(24):8838-43. PubMed PMID: 24876273; PubMed Central PMCID: PMC4066479.

d. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, **Chen Y**, Mohammad TA, Chen Y, Fedor HL, Lotan TL, Zheng Q, De Marzo AM, Isaacs JT, Isaacs WB, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014 Sep 11;371(11):1028-38. PubMed PMID: 25184630; PubMed Central PMCID: PMC4201502.

3. We have **applied our novel methodologies** (differential expression, gene association network, gene set enrichment) **to cancer studies**, such as liver cancer.
   a. Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, Ambs S, **Chen Y**, Meltzer PS, Croce CM, Qin LX, Man K, Lo CM, Lee J, Ng IO, Fan J, Tang ZY, Sun HC, Wang XW. MicroRNA expression, survival, and response to interferon in liver cancer. N Engl J Med. 2009 Oct 8;361(15):1437-47. PubMed PMID: 19812400; PubMed Central PMCID: PMC2786938.
   b. Sumazin P, **Chen Y**, Trevino LR, Sarabia SF, Hampton OA, Patel K, Mistretta TA, Zorman B, Thompson P, Heczey A, Comerford S, Wheeler DA, Chintagumpala M, Meyers R, Rakheja D, Finegold MJ, Tomlinson G, Parsons DW, Lopez-Terrada D. Genomic analysis of hepatoblastoma identifies distinct molecular and prognostic subgroups. Hepatology (Baltimore, Md.). 2017; 65(1):104-121. PubMed PMID: 27775819
   c. Comerford SA, Hinnant EA, **Chen Y**, Bansal H, Klapproth S, Rakheja D, Finegold MJ, Lopez-Terrada D, O'Donnell KA, Tomlinson GE, Hammer RE. Hepatoblastoma modeling in mice places Nrf2 within a cancer field established by mutant b-catenin. JCI insight. 2016; 1(16):e88549. PubMed [journal] PMID: 27734029, PMCID:PMC5053152
   d. Hsiao TH, Chen HI, Lu JY, Lin PY, Keller C, Comerford S, Tomlinson GE, **Chen Y**. Utilizing signature-score to identify oncogenic pathways of cholangiocarcinoma. Transl Cancer Res. 2013 Feb 1;2(1):6-17. PubMed PMID: 23905013; PubMed Central PMCID: PMC3725832.

4. We also **pilot genetic regulatory and interaction network studies** when gene-wide expression profiling technique become matured. From earlier model of co-determination network and to recent regulation by using competitive endogenous RNA (ceRNA) concept.
   a. Kim S, Dougherty ER, **Chen Y**, Sivakumar K, Meltzer P, Trent JM, Bittner M. Multivariate measurement of gene expression relationships. Genomics. 2000 Jul 15;67(2):201-9. PubMed PMID: 10903845.
   b. Meng J, Lu M, **Chen Y**, Gao SJ, Huang Y. Robust inference of the context specific structure and temporal dynamics of gene regulatory network. BMC Genomics. 2010 Dec 1;11 Suppl 3:S11. PubMed PMID: 21143778; PubMed Central PMCID: PMC2999341.
   c. Ma C, Chen HI, Flores M, Huang Y, **Chen Y**. BRCA-Monet: a breast cancer specific drug treatment mode-of-action network for treatment effective prediction using large scale microarray database. BMC Syst Biol. 2013;7 Suppl 5:S5. PubMed PMID: 24564956; PubMed Central PMCID: PMC4029357.
   d. Flores M, **Chen Y**, Huang Y. TraceRNA: a web application for competing endogenous RNA exploration. Circ Cardiovasc Genet. 2014 Aug;7(4):548-57. PubMed PMID: 25140062; PubMed Central PMCID: PMC4560354.

Complete List of Published Work: https://www.ncbi.nlm.nih.gov/myncbi/yidong.chen.1/bibliography/public/

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

Ongoing Research Support

2 P30 CA054174, NIH/NCI
Mesa, R. (PI)
08/01/20-07/31/25
Cancer Therapy and Research Center/Caner Center Admin

This is a P30 Cancer Center Support Grant at the University of Texas Health Science Center at San Antonio. This grant provides funding for the conduct of cancer clinical trials, prevention studies and assistance with statistical design and analysis of translational cancer research projects. Specifically, this is for support of the biostatistics and medical informatics core.
Role: KP

NIH/NCATS 1UL1 TR002645-01
05/24/2018 – 04/30/2023
Institute for Integration of Medicine & Science (IIMS): A Partnership to Improve Health
The mission of the IIMS is to achieve optimal integration of clinical and translational research, education, training, and career development across all UTHSCSA schools and among our partner organizations in the South Texas region.
Role: KP

1R01CA152063-01A1, NIH/NCI
Bishop, A (PI)
09/01/11-08/30/16 (no cost extension)
Improving etoposide treatment of Ewing's sarcoma
To identify genes involved in surviving etoposide exposure and to determine the utility of targeting these genes to improve etoposide treatment of Ewings sarcoma.
Role: KP

R01 CA192564-01A1, NIH/NCI
Sun, L (PI)
09/01/15 – 08/31/20 (NCE)
Aging mammary stem cells and breast cancer prevention
The major goals are to investigate the potential utility of Rapamycin and other anti-inflammatory agents for the prevention of the transformation of murine and human mammary stem cells.
Role: KP

CPRIT RP160732, Cancer Prevention & Research Institute of Texas
Chen, Yidong (PI)
01/08/2016 – 30/28/2021
Title: UTHSCSA Cancer Genome Sequencing and Computation Core (UTHSCSA-CGSCC)
Summary: To upgrade and expand our current genome sequencing facility, and further accelerate the adoption of high-throughput technology in translational cancer genetics and epigenetics research at UTHSCSA and the South Texas region.

1 R01 CA205965-01A1, NIH/NCI
Curiel, T (PI)
06/01/2017 – 5/31/2022
Title: Novel Tumor Intrinsic PD-L1 Signals Direct Tumor Immune Cell Infiltration
Summary: The project focuses on melanoma to study how tumor PD-L1 alters tumor immune infiltrates and immunotherapy responses. Specifically, the study will study differential treatment outcomes by tumor PD-L1 status (control versus PD-L1lo (shRNA) B16), examine tumor PD-L1-driven mTOR signal effects on TIL and immunotherapy responses, and specify cell-intrinsic PD-L1 effects in human melanoma.
Role: CoI

CPRIT RP190346, Cancer Prevention & Research Institute of Texas
Chen, Yidong (PI)
09/01/2019 – 08/31/2022
Title: Predicting drug response from genomic data using deep learning methods
Summary: we propose a study with a new deep learning model termed “Supervised Adversarially Learned Inference” (SALI) and a unique “transfer learning” module to transfer learned knowledge from cancer cell-lines to patient tumor cells, a major effort to bring *in vitro* experiment results to actual tumor samples.

1 R01 CA241554-01A1, NIH/NCI
Bishop, Alexander, PI

5/01/2020 – 4/30/2025
Dysregulated Transcription Processes in Ewing Sarcoma
Summary: The project will test the hypothesis that Ewing sarcoma is dependent upon RNA processing machinery to prevent accumulation of toxic R- loops. We will investigate the mechanistic relationship between transcription levels, R-loops and splicing in EwS; examine whether EwS is particularly reliant on splicing components or RNA:DNA helicases to block toxic conversion of R-loops, and test efficacy of removing the R-loop metabolizing program to inhibit EwS tumor growth.
Role: KP

R01CA239227-01A1, NIH/NCI
Rao, Manjeet, PI, 12/01/2019 – 11/30/2024
Title: FoXM1 inhibition: a novel therapeutic avenue to treat breast cancers
Summary: The major goals of this project are to address the role of FOXM1 in regulating DNA repair events and targeting FOXM1 for treating breast cancer patients.
Role: KP

Completed Research Support (last three years)

RP120685-C2, Cancer Prevention & Research Institute of Texas (PI, Chen, Y) 08/01/12-02/30/19
Title: Functional Genomics and Computation Core
Project goal: A computational genomic core to perform integrative genomic analysis for a collection of soft-tissue sarcoma tumors from children's in Texas, and genomic data from model organisms.

NIH/NCATS 1UL1TR001120-01 (Investigator; PI, Clark, R.) 09/26/2013 – 04/30/2018
Institute for Integration of Medicine & Science (IIMS): A Partnership to Improve Health
Project goal: The mission of the IIMS is to achieve optimal integration of clinical and translational research, education, training, and career development across all UTHSCSA schools and our partner organizations.
Role: KP

CPRIT RP150445 (Investigator; PI, Bishop, A.) 02/18/2015 – 01/31/2018
Title: Ewings Sarcoma, a homologous recombination defective disease
Project goal: To examine the finding that Ewing's sarcoma cells are deficient in homologous recombination and the role in transcription progression and the impact on replication and recombination.
Role: KP

NIH/NIGMS 1R01GM113245-01, NIGMS/NIH (PI: Huang, Yufei) 09/01/2014 – 06/30/2017
Graphical models for characterizing global RNA methylation
Project goal: The goal of this project is to develop, for the first time, computational graphical models to enable 1) accurate and reproducible detection of global mRNA methylations, and 2) context-specific differential RNA methylations in normal and disease states.
Role: CPI

SALSI Innovation Challenge Program San Antonio Life Science Institute (PI) 05/01/2016 – 04/31/2017
A Cloud Computing Pipeline for Precision Medicine
Project goal: Utilizing the large data storage and cloud computing resource, we implement our customized WES and WTS analysis pipelines to cloud platform.

NIH/NCI P20 CA165589-01A1 (Investigator; PI, Leach, R.) 09/01/2012 – 08/30/2016
The Cancer Bioinformatics Initiative: A UTSA/UTHSCSA Partnership
Project goal: This program will provide opportunities for students and faculty at the University of Texas at San Antonio, a minority serving institution, to gain relevant experience by interacting directly with members at the Cancer Therapy and Research Center at the UT Health Science Center at San Antonio.
Role: co-PI

PHS Fellowship Supplemental Form

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Introduction

1. Introduction to Application (for Resubmission applications)	HM_F31_introduction1027949207.pdf

Fellowship Applicant Section

2. Applicant's Background and Goals for Fellowship Training*	HM_F31_background_training_goals1027949182.pdf

Research Training Plan Section

3. Specific Aims*	HM_F31_specific_aims1027949186.pdf
4. Research Strategy*	HM_F31_research_strategy1027949211.pdf
5. Respective Contributions*	HM_F31_respective_contributions1027949188.pdf
6. Selection of Sponsor and Institution*	HM_F31_selection_sponsor_institution1027949189.pdf
7. Progress Report Publication List (for Renewal applications)	
8. Training in the Responsible Conduct of Research*	HM_F31_responsible_conduct_research1027949193.pdf

Sponsor(s), Collaborator(s) and Consultant(s) Section

9. Sponsor and Co-Sponsor Statements	HM_F31_sponsor_statement1027949165.pdf
10. Letters of Support from Collaborators, Contributors and Consultants	Sinclair_support_letter1027949173.pdf

Institutional Environment and Commitment to Training Section

11. Description of Institutional Environment and Commitment to Training	NB_institution_environment1027949176.pdf
12. Description of Candidate's Contribution to Program Goals	

Other Research Training Plan Section

Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? ☐ Yes ☑ No

13. Are vertebrate animals euthanized?

If "Yes" to euthanasia
Is method consistent with American Veterinary Medical Association (AVMA) guidelines?

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

14. Vertebrate Animals

Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T17:49:31.000-05:00

PHS Fellowship Supplemental Form

Other Research Training Plan Information

15. Select Agent Research

16. Resource Sharing Plan HM_F31_resource_plan1027949201.pdf

17. Authentication of Key Biological and/or Chemical Resources HM_F31_authentication1027949200.pdf

Additional Information Section

18. Human Embryonic Stem Cells

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), using the registry information provided within the agency instructions. Or, if a specific stem cell line cannot be referenced at this time, please 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):

19. Alternate Phone Number:

20. Degree Sought During Proposed Award:

Degree:	If "other", indicate degree type:	Expected Completion Date (MM/YYYY):

21. Field of Training for Current Proposal*: 154 Molecular Biology

22. Current or Prior Kirschstein-NRSA Support?* ☐ Yes ☑ No

If yes, identify current and prior Kirschstein-NRSA support below:

Level*	Type*	Start Date (if known)	End Date (if known)	Grant Number (if known)

23. Applications for Concurrent Support?* ☐ Yes ☑ No

If yes, describe in an attached file:

24. Citizenship*

U.S. Citizen U.S. Citizen or Non-Citizen National? ☑ Yes ☐ No

Non-U.S. Citizen ☐ With a Permanent U.S. Resident Visa

☐ With a Temporary U.S. Visa

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: ☐

25. ☐ Change of Sponsoring Institution Name of Former Institution:*

PHS Fellowship Supplemental Form

Budget Section

All Fellowship Applicants:

26. Tuition and Fees*:

☐ None Requested ☑ Funds Requested

Year 1	$4,077.00
Year 2	$4,169.00
Year 3	$4,263.00
Year 4	
Year 5	
Year 6 (when applicable)	
Total Funds Requested:	$12,509.00

Senior Fellowship Applicants Only:

	Amount	Academic Period	Number of Months
27. Present Institutional Base Salary:			

28. Stipends/Salary During First Year of Proposed Fellowship:

	Amount	Number of Months
a. Federal Stipend Requested:		

	Amount	Number of Months
b. Supplementation from Other Sources:		

Type (e.g.,sabbatical leave, salary)

Source

Appendix

29. Appendix

Funding Opportunity Number: PA-21-051. Received Date: 2020-12-08T17:49:31.000-05:00

INTRODUCTION

Thank you for the detailed and valuable comments on my F31 fellowship submission. In responding to reviewer comments, the aims have undergone extensive revision, along with corresponding changes to the research strategy and project summary. As a result, two new letters of support are now included. Additionally, In the intervening months, I submitted a first-authored paper for publication, began preparing a new first-authored manuscript, was awarded a graduate fellowship, led a bioinformatics workshop, and began managing several research assistants. These changes are reflected in the application where appropriate. The primary objections of reviewers were **(C1)** the disjointed nature of the two aims, **(C2)** the over-ambitiousness of the proposal in total, and **(C3)** the lack of alignment between the research goals and the hypothesis. These critiques **(C1-3)** relate to fundamental flaws in my original aims, Aim 1O (original Aim 1) and Aim 2O (original Aim 2). To address these concerns, I restructured and streamlined these aims, leading to Aim 1N (new Aim 1) and Aim 2N (new Aim 2). Additionally, reviewers noted **(C4)** the potential insufficiency of mouse embryonic fibroblasts (MEFs) for Aim 2O and lack of support for the feasibility of the Aim 2O design. These critiques (and others) are addressed below.

Regarding (C1): It was noted that "the focus on two different biological processes would lead to superficial learning" (*Resume and Summary of Discussion*). **Response:** To address this vital concern, the aims were first re-structured to synergistically interrogate the role of R-loops in enhancer stability with epigenetic aging, and re-formulated so that both would use the same aging model (ICE MEFs). Aim 1N will elucidate both the molecular mechanism of R-loops in enhancer stability and the impact on aging phenotypes, recapitulating the value of Aim 1O and Aim 2O with a streamlined set of experiments. I also propose Aim 2N, which builds upon ongoing research to test the hypothesis that STAG2 protects R-loops to support enhancer stability with aging. Whereas the goal of Aim 1N is to elucidate the role of physiological R-loops within key enhancers that are lost with epigenetic aging, the goal of Aim 2N is to determine how the STAG2/R-loop interaction preserves youthful epigenomic stability genome-wide. Where Aim 1O and Aim 2O were disjointed, Aim 1N and Aim 2N are synergistic, with the potential to reveal a novel physiological role for R-loops in enhancer stability with aging.

Regarding (C2): It was noted that the "project was also overambitious for the timeframe" (*Resume and Summary of Discussion*). **Response**: In addressing **(C1)**, the aims were restructured such that fewer experiments with fewer optimization steps are needed. Rather than optimizing the CRISPR-based system for selectively degrading R-loops (RED-LasRR) in two unrelated cell lines, it is now only necessary to optimize RED-LasRR in ICE MEFs. Our laboratory has already optimized RED-LasRR in another cell line, and that experience will make the process of our optimization in MEFs more efficient. Furthermore, the number and complexity of experiments was unnecessary and has been trimmed down to the core experimental plan. Additionally, it is worth noting that my record shows I am highly productive and capable of quickly accomplishing goals which may typically take longer to complete. This is evidenced by the fact that, as a third-year PhD student, I have already accumulated several authorships, with one first authorship in press, another under review, and a third in preparation, all while managing volunteer research assistants and pursuing teaching/advocacy outside the lab.

Regarding (C3): It was noted that there was "a disconnect between the stated hypothesis and the research goals" (*Resume and Summary of Discussion*). **Response:** In restructuring the aims as noted above, this issue was also addressed. For example, the sub-hypothesis of Aim 1O proposed a mechanism related to RNA binding proteins which the experimental strategy did not address. Conversely, Aim 1N provides a sub-hypothesis regarding eRNA R-loops in enhancer stability which is directly tested by the experimental design.

Regarding (C4): It was noted that the "key reagent for the second aim which would be insufficient for the assays proposed" (*Resume and Summary of Discussion*). In particular, it was noted by Reviewer 2 that MEFs are quick to senesce and the feasibility of using RED-LasRR in these cells needed to be better supported. **Response**: Firstly, I have now included a letter of support from David Sinclair ensuring that he is willing to provide breeding age ICE/Cre mice when requested. Secondly, in June of this year, a study which utilized a nearly identical CRISPR-based construct for selectively degrading specific R-loops to influence a cellular phenotype (reprogramming to pluripotency) in non-immortalized MEFs was published, demonstrating the feasibility of using our proposed design. This is now described in the specific aims and research strategy.

Other critiques addressed: It was also noted that the *Biohazards* description was unacceptable because of the need for training to work with cell lines (Reviewer One). This is now addressed in *Facilities and Other Resources*. It was also deemed unacceptable because of the use of lentivirus proposed without IBC approval (Reviewer Three). However, neither aim now relies on the use of lentivirus in this resubmission. Additionally, it was noted that I have not had a publication thus far relating to the original experimental plan (Reviewer Two). Now, my middle authorship in *Nucleic Acids Research* is a direct contribution to the basis for Aim 2N.

APPLICANTS BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

Doctoral Dissertation and Research Experience

Undergraduate Research Scientist *Spring 2015 – Spring 2016*

Auditory cue absence as a conditioned stimulus for delay eyeblink conditioning

Research Advisor: Matthew Campolattaro, PhD, Christopher Newport University, Newport News, VA

As an undergraduate student double-majoring in neuroscience and philosophy, I was invited to participate in my first research experience during the spring of my junior year under the mentorship of Matthew Campolattaro, PhD. Dr. Campolattaro's research focus was the neuroanatomical basis of motor learning. It had been previously shown that the medial geniculate nucleus (MGN) is required for rats conditioned to blink in response to an auditory cue ("tone-on") to recall this conditioned response (i.e. conditioned responding). The hypothesis for my project was that the same pathway is required for conditioned responding to an auditory cue being removed ("tone-off"). I used stereotaxis surgery to cannulate several rats and mount a headstage for stimulating and measuring the eyeblink response. I then observed that, if the MGN was inactivated, the rat would be unable to demonstrate conditioned responding, demonstrating the importance of the MGN in tone-off motor learning. In this research, I learned multiple animal techniques, including stereotaxis surgery, post-op care, perfusion, and tissue extraction. I also learned how to consistently run rigorous behavioral trials and perform tissue histology. Though my contributions to this project were not deemed sufficient to warrant authorship, I was excited to see that the work was published. While these experiences occurred late in my undergraduate career, I felt energized by them and resolved to continue research at the graduate level.

- Campolattaro, M. M., Savage, S. W. & Lipatova, O. Auditory cue absence as a conditioned stimulus for delay eyeblink conditioning. **Behav. Neurosci**. 131, 149–154 (2017).

Master's Student Researcher *Spring 2017 – Fall 2017*

A 3D-printed microfluidic biochip for detection of exosomal miRNA biomarkers

Research Advisor: Yun Wu, PhD, SUNY Buffalo, Buffalo, NY

During my first year as a master's student, I took an interest in the development of biomarker detection tools, seeing them as a way to uncover early-stage cancers and improve patient survival. During my first semester, I learned to make microfluidic devices, and began learning 3D printing. Noticing these efforts, Dr. Yun Wu invited me to join her research group and develop a novel microfluidic, 3D-printed, biomarker-detecting biochip. In this research project, I created a biochip designed to increase the interaction efficiency of liposomal detectors and exosomal miRNA biomarkers for cancer screening. I cultured lung cancer and control cells, harvesting exosomes from each. I formulated liposomal detectors and validated them using nanoparticle tracking analysis (NTA). Then, I designed the biochip, 3D-printed the mold using stereolithography (SLA), and fabricated the silicon microfluidic channels. By running an exosome- and liposome-containing water phase through the biochip with an oil continuous phase, I was able to create bubbles of < 75μm in diameter. However, the small-scale mixing in these bubbles did not improve the exosome-liposome fusion efficiency. In analyzing the causes of this unexpected finding, I modeled the fluidic interactions within the biochip and determined that small-scale mixing would not provide any further benefit. From this short research project, I gained significant experience in nanomedicine research techniques which supported my transition to my ultimate master's research project.

Master's Student Researcher *Fall 2017 – Summer 2018*

Phosphatidylserine liposomes for the treatment of peanut hypersensitivity

Research Advisor: Jonathan Lovell, PhD, SUNY Buffalo, Buffalo, NY

Following the discontinuation of my previous project, I decided to switch labs to work alongside Dr. Jonathan Lovell with whom I was already a co-executive in the nanomedicine start-up he founded. Dr. Lovell, knowing my interest in formulating a novel nanomedicine, offered me the opportunity to work on a project in which I would develop a treatment for peanut allergies (hypersensitivity). Peanut hypersensitivity is the most common clinical presentation of severe food allergies, especially among children at incidence rates of 3-8%. In this project, I created a novel liposomal nanomedicine designed to treat peanut hypersensitivity by inducing antigen-specific immune tolerance towards peanuts. Using analytical approaches like UV/Vis spectroscopy, I validated the composition and size of the drug. Using ELISA and symptom assessment, I verified the successful induction of peanut hypersensitivity in the mice. These experiments provided me with experience in drug development, which I aim to use in the development of novel anti-aging therapeutics in my future research career. During this research experience, I also had the brief opportunity to explore my long-time intellectual interest in aging in a

research context. I was asked to develop a senolytic therapy which would target IL-6 using a similar liposome formulation to the peanut project. Though the IL-6 project never reached the bench, researching and planning it catalyzed my excitement for aging research and anti-aging therapeutic development. Shortly after, I applied for the Biology of Aging program at UT Health San Antonio.

Rotation Student *Fall 2018*

Pathogenic Tau Causes a Toxic Depletion of Nuclear Calcium

Research Advisor: Bess Frost, PhD, UT Health San Antonio, San Antonio, TX

Upon arriving at UT Health San Antonio (UTHSA), I was eager to find aging research experiences that would provide me an opportunity to refine my nascent bioinformatics skills. I rotated with Dr. Bess Frost because of her exciting work in tau and transposon biology, as well as her offer of an interesting dry-lab project addressing the role of calcium signaling in Alzheimer's Disease (AD). Dr. Frost's research had recently uncovered a link between pathological tau accumulation, loss of calcium signaling, and decreased CREB transcription factor binding. Therefore, it was hypothesized that tau's effects would be reflected by a dysregulation in transcription of CREB gene targets. To test this hypothesis, I obtained CREB binding targets by mining publicly available CREB ChIP-sequencing datasets and performing a genome-wide search for the CRE motif to which CREB binds. I then analyzed the differential gene expression between tau-expressing transgenic flies and their wildtype counterparts, demonstrating that these CREB targets were differentially expressed with the aberrant accumulation of tau. This provided the data necessary to help support this group's recent publication in Cell Reports and offered strong evidence for the central role of calcium in AD. The experience also gave me an opportunity to greatly hone my nascent bioinformatics skills in answering an exciting biological research question. While I no longer research Alzheimer's disease, it was this rotation experience which first challenged me to understand not only how to perform an analysis, but to also understand the analysis at a mathematical level and intelligently optimize it. It is this deeper knowledge which today enables me to perform complex integrative bioinformatics analyses, such as those which I have proposed in my research aims.

- Mahoney, R., Ochoa Thomas, E., Ramirez, P., **Miller, H.**, Beckmann, A., Zuniga, G., Dobrowolski, R., & Frost, B. Pathogenic Tau Causes a Toxic Depletion of Nuclear Calcium. **Cell Rep**. 32, 107900 (2020).

Other projects: sex- and species-specific differences in transposon expression, RNA-seq analysis optimization, transposon identification algorithm benchmarking.

Rotation Student and PhD Student *Fall 2018 - Present*

The dynamics and impact of R-loops in epigenetic stability and aging

Research Advisor: Alex Bishop, DPhil, and Yidong Chen (Co-mentor), PhD, UTHSA, San Antonio, TX

While I was eager to improve my bioinformatics skills, I was also determined to gain equal training in molecular biology techniques. In seeking a mentor who would be interested in meeting these diverse training goals, I reached out to Dr. Bishop and was invited to rotate in his lab. My rotation project involved the application of both wet- and dry-lab techniques to address the question of how cell lines derived from patients with Ewing sarcoma, a pediatric bone cancer, differ from one another. First, I grew several Ewing sarcoma cell lines, isolated RNA for sequencing, and then analyzed the data. Curiously, I observed that the cell lines were heterogenous in their expression of gene targets for their driver oncogene, EWSR1-FLI1, and that this corresponded to the expression of genes involved in epithelial-to-mesenchymal transition (EMT). These findings suggested a possible mechanism of Ewing sarcoma EMT via downregulation of its driver oncogene. Furthermore, I gained valuable experience in utilizing wet- and dry-lab skills in the completion of a cogent research project, experience which will enable the successful completion of the wet- and dry-lab experiments in my proposed research strategy.

In order to boost my informatics skills, I also undertook a second rotation jointly between Dr. Bishop's laboratory and the laboratory of Yidong Chen, his bioinformatics collaborator. In this rotation, I was tasked with benchmarking splicing analysis algorithms. The accurate quantification and analysis of RNA splicing from sequencing data is challenging because most algorithms either rely upon transcript quantification or exon/intron quantification, each of which has different drawbacks and advantages. I observed that algorithms which relied upon the quantification of whole RNA transcripts were much faster but produced many false positives, and that the exon/intron-based algorithms were slower but produced more consistent results. It was this research in which I learned how to benchmark algorithms to determine appropriate software for a project. As my proposed aims rely upon complex integrative analyses, this experience will enable my success in completing them.

Following these research experiences, I decided to join Dr. Bishop's lab with Dr. Chen as my co-mentor. I consider this combination of mentors ideal for training me to become an independent researcher who can

leverage both wet- and dry-lab skills to ask pertinent biological questions. In the two years which have passed, both mentors have proved exceptionally dedicated to providing this training. Dr. Bishop has committed himself to my training as a biologist who can translate an abstract biological question into a concrete experimental plan. As a member of his lab, I have taken on multiple projects that have deepened by knowledge of biology and bioinformatics. Furthermore, Dr. Chen has repeatedly taken time out of his busy schedule to assist me with guidance in bioinformatics, continually demonstrating his dedication to my development as a bioinformatician through his active mentorship. The support of these mentors will be instrumental in the completion of my proposed project aims and my continued PhD training as a whole.

As a PhD student in the lab of Dr. Bishop and co-mentored by Dr. Chen, I have enjoyed the opportunity to pursue multiple exciting research interests in R-loop-biology, cell systems modeling, and aging. These projects have prepared me to undertake my proposed dissertation project: “The dynamics and impact of R-loops in epigenetic stability and aging”. Here is a brief description of these projects and their relationship to my proposed research aims and long-term research interests in aging biology:

A cell “state” is a specific pattern of gene expression that ultimately controls cell behavior and identity. Cellular states can be elucidated from high-throughput sequencing data to uncover novel insights into complex biological systems. In my research career, I will aim to elucidate the types of cell states which exist, the state transitions which cells can undergo, and the impact of aging upon these networks. In the pursuit of this interest, I first created a systems-level model of cell state transitions in Ewing sarcoma. This analysis revealed novel requirements for normal tissues to transform into Ewing sarcoma, helping to elucidate the search for Ewing sarcoma’s cell of origin, and eventually being published in *Cancers*. I also have a second manuscript under review based on a novel approach to analyzing gene co-expression correlations. This research has revealed tissue-specific co-expression correlation networks and provides a useful and user-friendly way to access gene co-expression correlation data. Additionally, in a manuscript currently under revision, I used this approach to reveal the antagonistic relationship between ATM and CD98 in the control of cell metabolism. Finally, I am working with a collaborator, Smita Krishnaswamy, to develop tools for assessing cell states and cell state transitions within large-scale datasets. From these studies, I have developed my ability to parse large sequencing datasets using mathematically advanced algorithms, revealing detailed insights about cell states and cellular networks in complex biological processes. It is this same systems-level knowledge which I will apply to meet the challenge of modeling aging cell systems in my proposed project and future career.

R-loops are three-stranded genomic structures caused by the hybridization of RNA to DNA. Typical R-loop research is concerned with unscheduled, pathological R-loops which are a source of genomic instability. However, R-loops have fundamental physiological roles in the epigenome that are often overlooked. For my proposed research project, I hypothesize that R-loops play a key role in aging through their interactions with enhancers, epigenomic elements which control gene transcription. To assess R-loops in this physiological role, it is necessary to develop a deeper understanding of R-loops and the aging systems they interact with. Thus far, I have engaged with two collaborators, Ashok Venkitaraman and Hong Wang, to analyze R-loop interactions in the biological systems they study. I have also engaged with Frederic Chedin in the development of a best-practices R-loop map processing pipeline that will clarify the data presented thus far in the field and am developing an R-loop classification algorithm and database (manuscript in preparation). These resources will greatly improve the clarity of my analysis of R-loop dynamics in my proposed research aims.

In the direct pursuit of my dissertation project, I have already analyzed the currently available data for the cellular model proposed in my Aim 1, revealing likely eRNA R-loops which participate in enhancer activation at key loci. Regarding Aim 2, the study with Hong Wang published in *Nucleic Acids Research*, on which I am an author, directly supports the mechanism of STAG/R-loop interaction that is proposed. Thus far, I have observed strong evidence for an interaction between R-loops, enhancers, and aging which I aim to elucidate further in completing my research aims. I have also gained valuable insight in combining bioinformatics and bench techniques to answer complex biological questions at a systems-level, a key facet of my long-term research interests which will continue to feature largely in my research training.

- **Miller, H. E.**, Gorthi, A., Bassani, N., Lawrence, L. A., Iskra, B. S., & Bishop, A. J. R. Reconstruction of Ewing Sarcoma Developmental Context from Mass-Scale Transcriptomics Reveals Characteristics of EWSR1-FLI1 Permissibility. **Cancers**. 12, 948 (2020).
- Pan, H., Jin, M., Ghadiyaram, A., Kaur, P., **Miller, H. E.**, Ta, H. M., Liu, M., Fan, Y., Mahn, C., Gorthi, A., You, C., Piehler, J., Riehn, R., Bishop, A. J. R., Tao, Y. J., & Wang, H. Cohesin SA1 and SA2 are RNA

binding proteins that localize to RNA containing regions on DNA. **Nucleic Acids Res**. 48, 5639–5655 (2020).

- **Miller, H. E.** & Bishop, A. J. R. (2020). Correlation AnalyzeR: functional predictions from gene co-expression correlations. (**under review**)
- **Miller, H. E.**, Hartano, S., Gorthi, A., Chedin, F., Bishop, A. J. R. (2020). RSeq: a best-practice pipeline for R-loop mapping. **(in preparation)**
- Iskra, B., Davis, L., **Miller, H. E.**, Chiu, Y. C., Bishop, A. R., Chen, Y., & Aune, G. J. (2020). Assessing the heterogeneity of cardiac non-myocytes and the effect of cell culture with integrative single cell analysis. BioRxiv. https://doi.org/10.1101/2020.03.04.975177 (**pre-print**)
- Romero, J.C., Tonapi, S., Loranc, E., Bassani, N., Lawrence, L.A., **Miller, H.E.**, Robledo, D.G., Cao, L., Nie, J., Kanda, K., Gorthi, A., Lane, A.N., Fan, T.W.N., Zha S., Musi, N., & Bishop, A. J. R. (2020). ATM protein kinase regulates CD98HC dependent antiport activities to control cell metabolism. (**under revision**)

Training Goals and Objectives

My long-term goal is to be a leading academic researcher in the aging biology field. My primary research interest involves comprehensively understanding the aging epigenome at a systems level. This research goal necessitates elucidating the impact of aging upon cell state dynamics and the contribution of metabolic, proteomic, autophagic, and other processes. It also necessitates developing a dynamic network model of these systems and their interactions, preferably in a form that is accessible for non-computational researchers to use. Success in these aims requires an understanding of the biology and the research methods, as well as advanced computational aptitude. Additionally, I have a strong interest in mentoring and teaching students so that they can prepare for research careers of their own. My goal for this fellowship is to build the skills and acquire the knowledge necessary to accomplish these goals, advancing the aging biology field, and helping to train the next generation of scientists. I have worked alongside my mentors to develop the following training goals:

Build a strong bench research skillset: Through my coursework in biomedical sciences and several wet-lab research experiences, I have a good understanding of what will be required to undertake my proposed research. I will continue to develop my lab bench skills to complete my proposed research project and to meet my career goals. I expect that my training in Dr. Bishop's lab will improve my general cell and molecular skills, including plasmid manipulation, qPCR, various cellular biology methods, and state-of-the-art techniques like DNA:RNA immunoprecipitation (DRIP) sequencing and sgRNA-guided CRISPR.

Strengthen my computational expertise: Though my background in engineering has prepared me to perform computational research, it is vital that I continue to refine my fundamental bioinformatics skills. My co-sponsor, Dr. Yidong Chen, is a leading expert in the application of machine learning to the computational study of biological systems who has already provided computational training to me through coursework and one-on-one mentorship. I have attended and will continue to attend advanced informatics classes at UTHSA to further strengthen these capabilities, including an advanced bioinformatics course, "NGS Data Analysis (INTD 6062)", which I plan to take next spring. I also attend his bioinformatics journal club, a forum for discussing current topics in computational biology, and deep learning journal club, a forum for discussing ongoing deep learning research and relevant applications in genomics. Furthermore, I am collaborating with Smita Krishnaswamy, a leading expert in manifold learning, an emerging area of applied mathematics, and will attend her "Machine Learning for Single Cell Analysis" workshop to deepen my skills in bioinformatics and mathematics.

Learn to conduct independent research: To meet my long-term goal of becoming an independent researcher in the field of aging biology, it is essential that I gain fundamental skills in designing research projects, preparing manuscripts, and grantsmanship. Dr. Bishop and Dr. Chen both challenged me to design the research aims which are proposed here, considering appropriate controls, replicates, alternatives, and next steps. Though they will continue to provide guidance, I will be pursuing these research aims independently. Additionally, Dr. Bishop and Dr. Chen have supported and encouraged me to draft manuscripts, such as the *Cancers* publication previously mentioned, as well as grants so I can develop my scientific writing skills and begin a track record of research funding which will eventually lead to research independence.

Teach and mentor students: Through my experiences in three tutoring positions, mentoring student and faculty entrepreneurs as a venture coach, and teaching piano lessons in my spare time, I have gained tremendous appreciation for the value of mentorship. From those experiences, I realized that in addition to undertaking excellent research, I would also like to inspire others through various teaching and mentorship opportunities. In pursuit of these interests, I am training several undergraduate and master's research volunteers in bioinformatics

and mentoring them as they assist with my research projects. Beyond one-on-one mentorship, my long-term goal includes being a professor, lecturing on topics like data science in aging research, and inspiring young students to pursue a scientific career. It was for this reason that I have volunteered to be a co-founder of the nascent Bioinformatics Interest Group (BIG club) at UTHSA. In this position, I designed and led a 14-week R-based bioinformatics workshop this Fall which featured several faculty lecturers and was attended by dozens of students, a workshop series which we plan to expand in the Spring. I am also volunteering as the student representative on the bioinformatics curricula committee at UTHSA. In this position, I designed a graduate bioinformatics certificate program which the graduate school has now offered their informal approval of. With the help of Evelien Bunnik, an excellent computational biology faculty member at UTHSA who has agreed to be the first program director, we are planning formal submission of this certificate program for institutional approval in the coming months. Excitingly, this proposal has already yielded a new course (under formal review) which will teach R and Linux fundamentals to students who lack a computational background. I plan to also take two courses, “Introduction to Science of Teaching (INTD 6011)” and “Supervised Teaching (CSAT 6071)” which will provide hands-on training in designing and teaching my own course for first-year medical students.

Manage student researchers: With my long-term goal of running an academic research laboratory and mentoring students, it is vital that I develop my ability to manage student researchers. While at SUNY Buffalo, I managed 30 students as an engineering school project manager, an 8-member venture coaching team, and a 7-person biomedical startup. In these experiences, I learned that managerial skills such as project planning, communications, recruitment, and conflict resolution are paramount to the success of any team endeavor. Therefore, I aim to continue developing these skills as I train for a career as an independent researcher with my own laboratory. I have continued this training at UTHSA by recruiting and managing several undergraduate and master’s students who are working remotely on various bioinformatics research and software development projects. Team communications proceeds through Slack and Zoom, and we are also using the GitHub project management framework. Furthermore, I will be assigned a new PhD student to assist me with my bench research aims in the final year of my project (typical for the Bishop lab to maintain expertise and promote the next generation of researchers in the lab). These experiences will bolster my managerial skills and prepare me to achieve my long-term goal of running my own academic research group.

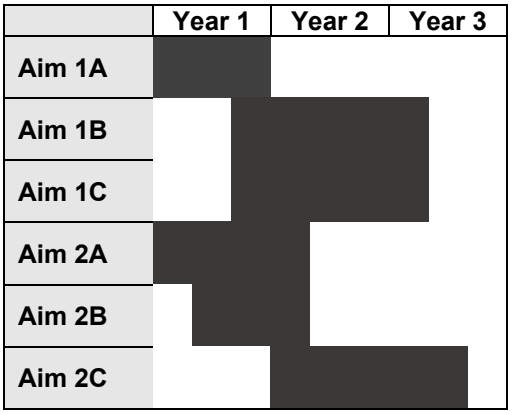
Develop my communications and presentation skills: With my goal of becoming a leader in the field of aging research, it is necessary for me to develop my ability to communicate complex scientific knowledge to my colleagues and the public at large. As a graduate student, I have been afforded multiple opportunities to refine my communication skills through weekly lab meetings, research presentations to our institute, and journal club presentations. During my training, I will pursue additional opportunities to develop my oral presentation skills, by competing in 3-Minute Thesis as well as presenting my research at national and international conferences (listed below) with posters and talks. I will further develop my writing skills by continuing to publish original research and by writing review articles. I have already begun planning one such article, to be written by myself alongside Aparna Gorthi, a post-doc in our lab. Additionally, I will continue developing my skills in grantsmanship by attending a regular workshop, “F-Troop”, designed to teach the skills needed to write NIH grants. Of note, my mentor, Dr. Bishop, takes great pride in developing the presentation skills of his mentees and spends significant time with each of us refining our presentations. With this support, I will continue to develop both my communication and presentation skills to help me become a successful independent researcher.

Activities Planned Under This Award

Activities		%		
		Y1	**Y2**	**Y3**
Course Work*	Research (IBMS 6097)	71.5	67	-
	Dissertation (IBMS 7099)	7	7	65
	NGS Data Analysis (INTD 6062)	2.5	-	-
	Introduction to Science of Teaching (INTD 6011)	-	1	-
	Supervised Teaching (CSAT 6071)	-	-	5
Seminars / journal clubs	Biology of Aging Journal Club (weekly)**	2	2	2
	Deep learning Journal Club (bi-weekly)**	1	1	1

	Bioinformatics Journal Club (bi-weekly)**	1	1	1
	Barshop Institute Seminars***	2	2	2
	Spotlight on Research Integrity (monthly)****	0.5	0.5	0.5
Meetings	Lab (weekly)	4	4	4
	Mentors (monthly)	0.5	0.5	0.5
	Dissertation Committee#	0.5	0.5	0.5
Conferences	Local##	2	2	2
	American Aging Association Annual Meeting	-	2	-
	Gordon Conference on Aging	-	-	2
Other	GCCRI Retreat (annual)	0.5	0.5	0.5
	Cell Systems and Anatomy Dept. Annual Retreat	0.5	0.5	0.5
	Machine Learning for Single Cell Analysis workshop	1	-	-
	Bioinformatics Interest Group meetings	1	1	1
	Bioinformatics Curriculum Committee meetings	0.5	0.5	0.5
	F-Troop	2	2	2
	Dissertation Defense###	-	-	5
	Manuscript Preparation	-	5	5
	Total	100	100	100

* Research (**IBMS 6097**) is taken every semester and receives a grade of Satisfactory, Unsatisfactory, or Honors based on recommendations by the supervising professor and dissertation committee and determined by performance in the laboratory. Dissertation (**IBMS 7099**) is taken for every semester following approval of the dissertation proposal. A minimum two semesters are required for graduation.



** The Biology of Aging journal club meets weekly and is led by Dr. Karl Rodriguez of the Barshop Institute. The Bioinformatics journal club and deep-learning journal clubs meet bi-weekly and are led by Dr. Yidong Chen of the GCCRI. In these journal clubs, students present original research articles with a substantial impact in aging, machine learning, and computational biology.

*** As part of the Biology of Aging program, I am expected to attend the Barshop Seminar series and write a critical review of each presentation. These seminars feature world-class aging researchers presenting work which is often still in progress.

**** Spotlight on Research Integrity is a monthly workshop organized by the institutional training grant directors at UTHSA. Lectures cover topics such as conflicts of interest, research compliance, record keeping, reproducibility, peer review, and data management.

The Dissertation Committee is comprised of 5 faculty at UTHSA and 1 external member. It will meet once per semester to assess the student's progress. My external committee member is Dr. Frederic Chedin (UC Davis), who has expertise in R-loop biology and R-loop mapping approaches.

Local conferences include the Frontiers of Translational Science conference, Mikiten Graduate Research Symposium, Barshop Conference on Aging, and the GCCRI Research Symposium.

The dissertation defense involves a written dissertation, a public seminar, and an oral examination for the Dissertation Committee, with less than two negative votes from committee members.

SPECIFIC AIMS

A growing body of evidence supports the central role of epigenetics in aging. Recent studies have shown that epigenetic changes accurately predict chronological age[1] and that epigenetic reprogramming prolongs lifespan in progeroid mice[2]. Most recently, it was found that epigenetic reprogramming can restore vision to aged mice with nerve degeneration or glaucoma[3]. These findings suggest a central role for epigenetic dysregulation in aging ('epigenetic aging'), though the underlying mechanisms and appropriate therapeutic targets remain enigmatic.

In a recent pair of preprints, the David Sinclair group introduced a mouse model with an inducible system for creating non-mutagenic, enzyme-induced double-strand breaks, "inducible changes to the epigenome" (ICE) mice[4,5]. They demonstrated that ICE mice display accelerated aging phenotypes at an organismal level and within ICE-mouse-derived embryonic fibroblasts (ICE MEFs)[4,5]. Interestingly, ICE MEFs show increased cellular plasticity with loss ("smoothing") of enhancers, genomic elements that drive gene expression via 3D chromatin interactions[5], important for MEF identity[5]. This suggests epigenetic aging may result from enhancer instability, yet the mechanisms by which enhancers are maintained in the "youthful" epigenome remain poorly understood.

Recent evidence has demonstrated that enhancers depend upon the interaction of co-factors with enhancer RNA (eRNA)[6,7], a long-noncoding RNA found within the enhancer complex[8]. Curiously, eRNA hybridizes with enhancer DNA to form a structure called an "R-loop" (an RNA:DNA hybrid with a displaced ssDNA strand)[9–13]. While previous evidence has documented important physiological roles of R-loops in other aspects of epigenomic maintenance[12,14–16], the role of eRNA R-loops within the enhancer remains enigmatic.

With Hong Wang (collaborator), we recently found that STAG2, a cofactor which supports lineage-specific enhancers[17–19], binds R-loops, and that STAG2 and R-loops colocalize at enhancers across the genome[16]. In unpublished results, we found that STAG2 protects R-loops from degradation by the RNaseH1 endonuclease. Additionally, from an analysis of RNA-Seq data in ICE mice, I found that DHX9, a protein which regulates R-loop formation[20,21], is the top over-expressed gene in ICE mouse muscle[4]. Finally, by re-analysis of ICE MEF ChIP-Seq data[5] along with public datasets[22,23], I found evidence of eRNA R-loops occupying MEF identity enhancer regions that are "smoothed" in ICE MEFs. Taken together, these findings suggest a key role for physiological eRNA R-loops and STAG2 in preserving enhancer stability with aging. Therefore, ***I hypothesize that dysregulation of physiological R-loops drives epigenetic aging by impairing youthful enhancer stability.***

Aim 1: Elucidate the mechanism of eRNA R-loops in enhancer stability with epigenetic aging. Hypothesis: *eRNA R-loops promote enhancer stability at loci important for epigenomic longevity.* To address this hypothesis, I will **(1A)** identify eRNA R-loops which correlate with enhancer dysregulation in ICE MEFs using R-loop profiling and integrative analysis with the previously-published epigenomic profiling data in ICE MEFs[5], **(1B)** test the effect of manipulating (protecting/degrading) specific eRNA R-loops (using CRISPR-directed RNaseH1, aka "RED-LasRR") on the stability of the enhancer which the eRNA R-loop resides within ("host enhancer") via assessment of enhancer-promoter looping and target gene transcription, and **(1C)** similar to Aim 1B, use RED-LasRR to determine whether manipulating eRNA R-loops can modulate (prevent or induce) cellular aging phenotypes (e.g., sensitivity to camptothecin) as previously demonstrated in ICE and Cre control MEFs[5].

Aim 2: Determine the impact of the STAG2/R-loop interaction in preserving the youthful epigenome. Hypothesis: *STAG2 protects R-loops to support enhancer stability and preserve the youthful epigenome*. To address this hypothesis, I will **(2A)** test the effect of manipulating STAG2 and R-loops on cellular aging phenotypes of ICE and Cre MEFs (similarly to Aim 1C), **(2B)** test the effect of manipulating STAG2 and R-loops on the development of epigenomic noise in ICE and Cre MEFs using ChIP-Sequencing for markers (e.g., H3K27me3) which are "smoothed" with epigenetic aging[5], and **(2C)** determine the effect of epigenetic aging in ICE MEFs on STAG2 binding at enhancers and across the genome using ChIP-Sequencing.

Significance: While R-loops have been studied in the context of aging before, these studies have only examined "pathological" R-loops (unscheduled R-loops which induce genomic instability)[24–28]. Consequently, the role of physiological R-loops in aging remains unexplored. Furthermore, no studies to date have examined the role of eRNA R-loops and their interactions with co-factors like STAG2 in enhancer dynamics. The current proposal addresses both of these important knowledge gaps. Finally, R-loops may represent an important sequence-specific drug target for future epigenetic therapies, indicating the importance of studying these mechanisms.

Innovation: These aims will utilize RED LasRR, an innovative new system for selective protection or degradation of specific R-loops, to test the causal relationship between R-loops and enhancer stability. Furthermore, these aims implement cutting-edge bioinformatics approaches, including an R-loop mapping/QC pipeline I recently developed (manuscript in preparation). **Training Relevance:** The completion of these proposed aims will ensure the applicant receives comprehensive training which prepares them for an independent research career in aging.

RESEARCH STRATEGY

A. Background and Significance

Epigenetic dysregulation as a cause of aging

The "epigenome" is the multi-tiered network of proteins, nucleotides, and metabolites which controls gene transcription and defines cell identity. A major component of this network involves DNA methylation, an epigenetic mark which typically represses gene transcription[29]. First described by the Horvath group in 2013, multiple studies have found a striking correlation between DNA methylation at certain sites and chronological age[1,30] and, moreover, that anti-aging interventions can reverse this "epigenetic clock"[31,32]. Another recent study demonstrated that chronological age correlates with changes in the activity of enhancers[33], *cis* regulatory elements which establish 3D interactions with promoters, inducing the transcription of their target genes[6]. In 2016, *Ocampo et al.* found that cyclic treatment of progeroid mice with "Yamanaka factors" (OSKM) led to significant increases in lifespan and regenerative capacity[2]. These results were echoed in a recent *Nature* study which demonstrated that, when aged mice with optic nerve damage or glaucoma were treated with "OSK" (OSKM without c-Myc), the optic nerve was regenerated and their eyesight was restored[3]. These studies demonstrate that aging correlates with epigenetic changes and that the restoration of the 'youthful epigenome' is possible.

Epigenetic aging modeled in vivo and in vitro reveals a 'smoothed' enhancer landscape

The David Sinclair group recently developed a novel mouse model for studying epigenetic aging. The "Inducible Changes to the Epigenome" (ICE) mice and their derived mouse embryonic fibroblast cell line (ICE MEFs) were recently described in a pair of pre-prints that have both completed review for publication in *Cell*. The first, *Hayano et al.*, demonstrated that ICE mice accumulate epigenetic "noise" through the induction of non-mutagenic DNA breaks (via Cre induced *I-Ppo*I endonuclease), leading to a robust phenocopy of aging in an accelerated timespan[4]. The second, *Yang et al.,* demonstrated that the induction of epigenetic aging in ICE MEFs recapitulates cellular aging phenotypes, including sensitivity to DNA damaging agents, senescence, and accelerated methylation clock age[5]. Furthermore, they demonstrated that ICE MEFs display a 'smoothed' enhancer landscape, including a loss of enhancer loci responsible for preserving MEF identity[5]. One example of this at *Col1a1* is demonstrated in **Figure 3**. These findings indicate that epigenetic aging can be recapitulated at the cellular level in ICE MEFs and that enhancer stability may preserve the youthful epigenome.

Enhancer RNA (eRNA) is necessary for enhancer stability and forms R-loops

In 2010, *Kim et al.* discovered that enhancer chromatin is actively transcribed into a non-coding RNA called 'enhancer RNA' (eRNA)[8], and subsequent studies demonstrated that eRNA supports enhancer functionality[34–41]. Recent evidence shows that eRNA is bound by co-factors within the enhancer, and that this binding supports the stability of enhancer-promoter looping[6]. These findings implied that eRNA is structurally anchored to the chromatin in a manner that would allow for robust binding by enhancer co-factors. In fact, *Pefanis et al.* found in 2015 that eRNAs can hybridize with DNA at enhancers, forming an "R-loop"[13]. Of note, R-loops are three-stranded genomic structures formed from the hybridization of RNA to DNA, leading to a displaced ssDNA loop. R-loops are thermodynamically stable structures which often serve as binding sites in the chromatin[14]. Multiple subsequent experiments also demonstrated the existence of eRNA R-loops across the genome[9–12]. Additionally, several studies have identified R-loop protein interactors, a large number of which are enhancer co-factors[20,42,43]. Finally, a recent study involving our group showed that targeting R-loops in ribosomal DNA, using CRISPR-directed RNaseH1 (RED-LasRR), prevents 3D chromatin looping[44]. Taken together, these results suggest eRNA R-loops promote enhancer stability via their interactions with enhancer co-factors.

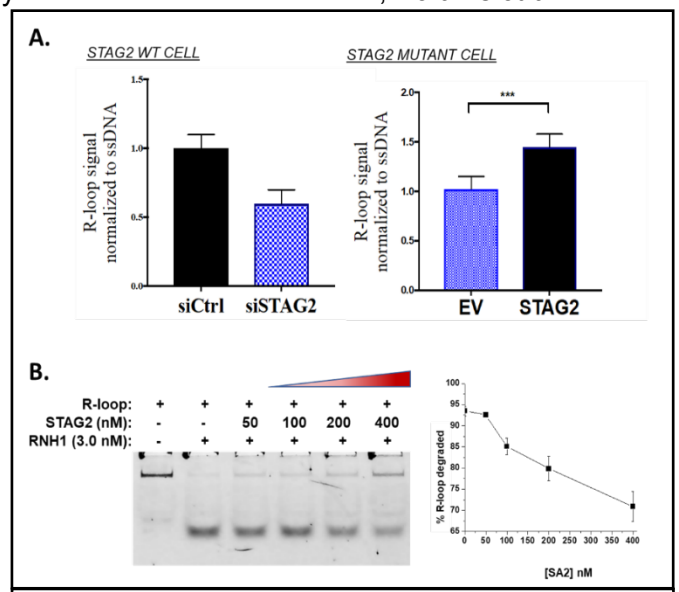


Figure 1: R-loops are protected by STAG2. (A) Knockdown of STAG2 decreased the R-loop signal in wildtype cells whereas over-expression of STAG2 in mutant cells increases R-loop levels. (B) STAG2 protects R-loops from degradation via RNaseH1 (RNH1).

STAG2/R-loop interactions and enhancer stability

STAG2 is a protein which supports looping of chromatin at enhancers, especially those which are crucial for lineage commitment and cellular identity[17–19], suggesting the possibility that STAG2 might help prevent the enhancer instability and loss of cellular identity ("plasticity") that

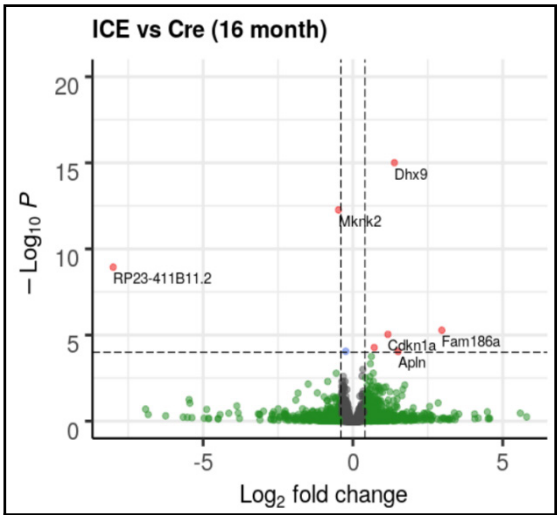


Figure 2: Differential gene expression in ICE vs Cre muscle. (Data source: *Hayano et al. 2019*)

characterizes epigenetic aging[5]. However, it was still unclear how this apparent role of STAG2 at the enhancer might relate to the proposed role of eRNA R-loops, which also appear to support enhancer dynamics. To understand the relationship between STAG2 and enhancer R-loops, we began by studying a pediatric bone cancer, Ewing Sarcoma, in which STAG2 loss of function mutations are the most common genetic event[45] and which is characterized by widespread dysregulation of enhancers[46] and R-loops[47]. Curiously, recent evidence had indicated that STAG2 mutations may enable greater cellular plasticity in these tumors[48]. Therefore, to better understand the relationship between STAG2, physiological R-loops, and enhancer stability, we engaged a collaborator, Hong Wang, who has significant expertise in biochemical methods. The resulting study on which I am an author, in *Nucleic Acids Research*, demonstrated that STAG2 binds R-loops with high specificity and that this happens at enhancers across the genome[16]. Moreover, from unpublished work resulting from our collaboration (**Figure 1**), we demonstrated in STAG2 mutant and wildtype cells, that manipulation of STAG2 leads to changing levels of R-loops (**Fig 1A**). To understand this phenomenon better we used an *in vitro* biochemical assay and demonstrated that STAG2 binds and protects R-loops from degradation by RNaseH1 (**Fig 1B**). Furthermore, from a re-analysis of ICE mouse RNA-Sequencing data[4], I found that DHX9, a protein which regulates R-loop formation[21], is the top over-expressed gene in ICE mouse muscle (**Figure 2**). Finally, I re-analyzed the H3K27ac ChIP-Sequencing data in ICE/Cre MEFs[5] along with R-loop profiling data (DRIPc) in 3T3 cells[22] (another embryonic mouse fibroblast cell line) and RNAPII ChIP-Sequencing in MEFs (Encode: ENCSR000CBX)[23]. Through integrative analysis, I identified multiple distal enhancers which were lost in ICE MEFs and which also had an eRNA R-loop. The *Col1a1* super enhancer was described by *Yang et al* as a key example of loss of MEF identity in ICE cells[5]. I demonstrate here that it also contains a prominent eRNA R-loop (**Figure 3**). Taken together, these findings have led to the current working model of this proposal: STAG2 binds and protects eRNA R-loops to prevent the loss of enhancer stability and cellular identity, safeguarding the integrity of the epigenome; a mechanism which I hypothesize is dysregulated with epigenetic aging (**Figure 4**).

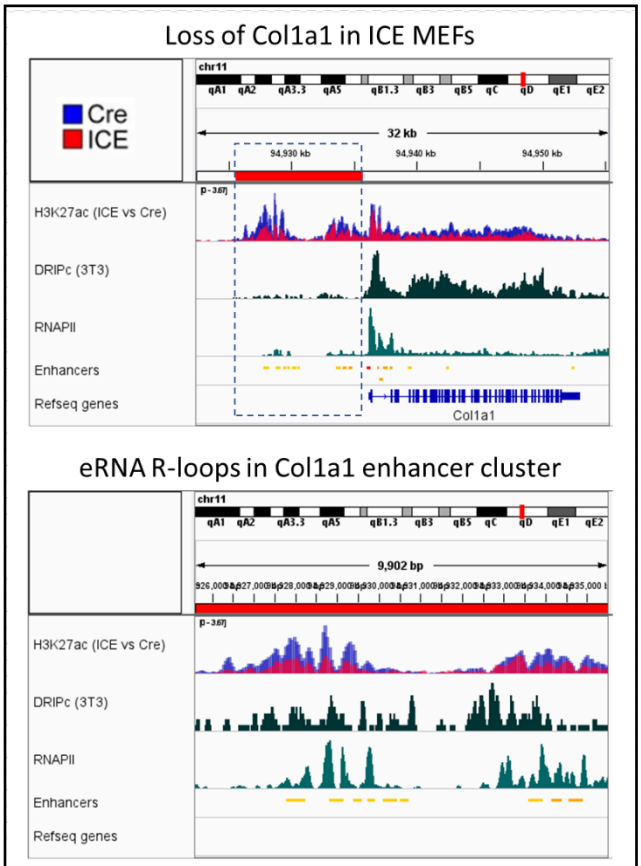


Figure 3: Loss of Col1a1 enhancer activity in ICE vs Cre controls. eRNA R-loops are found at the enhancer cluster of Col1a1. Lower panel is a closeup of the upper panel.

Significance

A new role for physiological R-loops in the epigenome: Physiological R-loops play crucial roles in the epigenome by promoting/terminating transcription[14], serving as binding sites for chromatin modifiers[15], and priming gene expression changes during cell reprogramming[12]. These aims will test the possibility that R-loops also promote enhancer stability, a novel role for physiological R-loops in the epigenome.

The first exploration of physiological R-loops in aging: Unscheduled R-loops can interfere with DNA replication and cause genome instability ("pathological" R-loops)[14]. Despite the clear importance of physiological R-loops, they remain sparsely studied compared to pathological R-loops. While pathological R-loops have been studied previously in the context of aging[24–28], the study proposed herein will be the first that explores physiological R-loops in the aging context.

A foundation for a novel class of therapeutics: R-loops are an attractive target for the development of novel therapeutics because of their sequence specificity and because they can be degraded or protected under different conditions. These aims propose to uncover R-loops which

could be targeted to treat epigenetic aging by restoring youthful enhancer stability, promoting human longevity.

B. Innovation

The innovation of this proposal is severalfold. First, the concept of our working model is novel – the idea that eRNA R-loops are necessary for enhancer function is innovative. Furthermore, the idea that aging may result from a dysregulation of the physiological role of eRNA R-loops is highly novel. In addition, the use of the RED-LasRR system will allow me to demonstrate a direct causal relationship between R-loops and enhancer stability. I will also utilize cutting-edge bioinformatics approaches in order to test the hypotheses in my aims. For example, I will use a processing pipeline for R-loop mapping which I recently developed (*manuscript in preparation*) which is highly innovative because it enforces several novel quality metrics and enables the classification of R-loops based on their associations with genomic features, gene expression, etc. Furthermore, the use of the ICE model system kindly provided by Dr. Sinclair is also highly innovative and allows a unique method to address the questions we ask in this proposal. Finally, if our hypothesis is proven correct, then it not only opens new avenues to explore with regard to R-loops and enhancer biology, but it also provides novel insight into aging itself and indicates the potential for R-loops as novel drug targets for future therapeutic interventions which restore the youthful epigenome (my long term research goal).

C. APPROACH

In these aims I will test the hypothesis that dysregulation of physiological R-loops drives epigenetic aging by impairing youthful enhancer stability. The proposed mechanism of this hypothesis is illustrated in **Figure 4**.

SPECIFIC AIM 1: *Elucidate the mechanism of eRNA R-loops in enhancer stability with epigenetic aging.*

Aim 1 Hypothesis: *eRNA R-loops promote enhancer stability at loci important for epigenomic longevity.*

Aim 1A. Identify eRNA R-loops which correlate with enhancer dysregulation in ICE MEFs

To find eRNA R-loops associated with epigenetic aging, I will utilize cells derived from the ICE mouse model described by *Hayano et al. 2019*[4]. ICE (Inducible Changes to the Epigenome) mice express an *I-PpoI*-based construct which induces double-strand breaks in non-coding DNA regions when exposed to tamoxifen (4-OHT)[4]. For this proposal Dr. Sinclair (see letter) provided me ICE (+ controls) mouse embryonic fibroblasts (MEFs) which display cellular aging phenotypes, such as accelerated methylation clock age (DNAmAge)[5].

To profile R-loop changes that correspond to enhancer dysregulation in ICE MEFs, an integrative sequencing and informatics approach will be used **(Figure 5)**. First, ICE MEFs and Cre controls will be induced as previously described[5] with media containing 0.5 μM 4-OHT for 24 hours. After 24 hours, the 4-OHT media will be replaced to halt induction. After 96 hours (recapitulating Sinclair's protocol), cells will be lysed and R-loop profiling via DRIP-sequencing will be performed as previously described[49]. Briefly, cells will be lysed, and genomic DNA will be extracted. A restriction enzyme cocktail will be used to digest DNA fragments overnight. S9.6 antibody (binds RNA:DNA hybrid >8nts) will be used to pull down R-loops using Dynabeads (Thermo). Finally, these samples will be submitted for paired-end 100bp sequencing on the Illumina HiSeq 3000. To address the limitations in the quality of previous R-loop mapping studies (*detailed in upcoming publication from Fred Chedin*), I will use the *RSeq* processing pipeline I developed (*manuscript in preparation*). Following best practices, reads will be filtered and trimmed using *fastp* [50] and aligned to the genome using *bwa mem*.[51] Peak calling will be performed with *MACS2*[52] and *EPIC2*[53], the resulting peaks will be integrated and annotated. Then, the *RSeq* QC model will be used to assess sample quality in relation to publicly available R-loop mapping data. *DiffBind*[54] will be used to calculate the differential R-loop signal between ICE and Cre groups and compared with enhancers found to be dysregulated in Sinclair's study (*Yang et al. 2019*) (eg. see Fig3).[5] This will allow

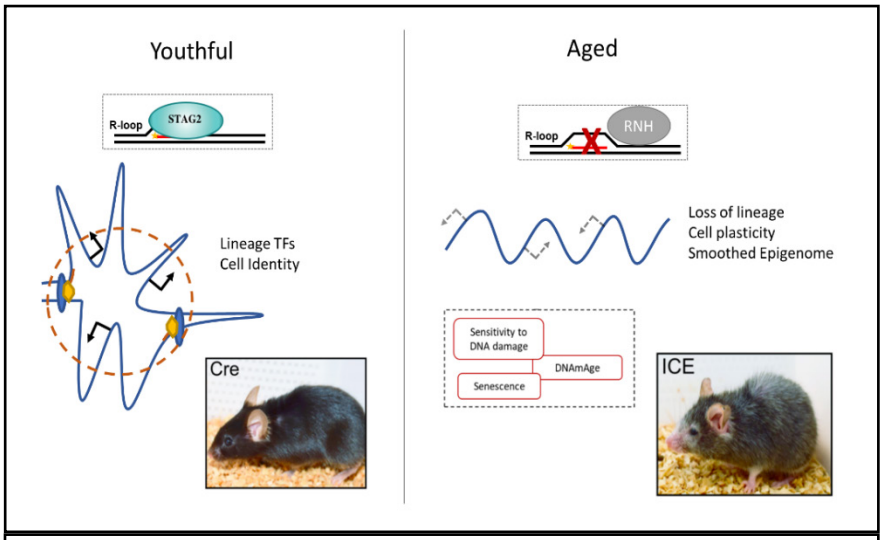


Figure 4: Working model: eRNA R-loops are bound by enhancer co-factors (e.g., STAG2) preventing their degradation, promoting enhancer stability at loci important for maintenance of cellular identity and prevention of epigenetic aging. (Images ICE/Cre mice 10 mo. after 4-OHT induction, from *Hayano et al.*)

the discovery of R-loops which correspond with enhancer dysregulation in ICE compared to Cre MEFs.

Pitfalls, alternatives, and additional controls: Epigenetic aging in ICE cells will be verified via senescence-associated beta-galactosidase (SABG) staining and camptothecin (CPT) treatment with western blotting for γH2AX and phospho-ATM to address the rigor and reproducibility of the previous results[5]. To ensure the efficiency of the S9.6 pulldown, DRIP-qPCR will be used as previously described[49]. Furthermore, it is unclear whether DRIP-sequencing will offer high enough resolution to enable comparison of our dataset with those previously published as this technique can be noisy and is not strand-specific. Therefore, if needed, I will implement qDRIP-sequencing, a new improvement upon DRIP-sequencing, that provides high-resolution, strand-specific R-loop maps[55]. Finally, the top eRNA R-loops will be confirmed using RT-qPCR for the eRNA moiety, coupled with DRIP-qPCR to confirm the R-loop. It may also be necessary to confirm key findings in both male and female MEFs.

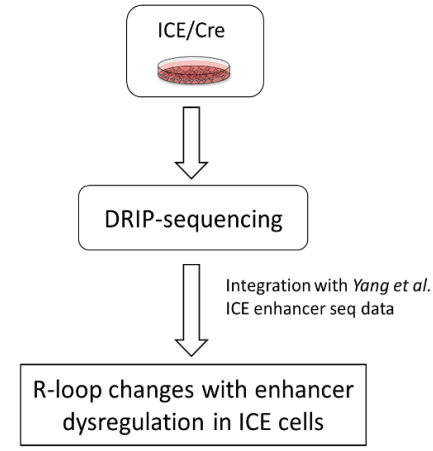


Figure 5: Aim 1A experimental strategy. R-loops will be mapped in ICE MEFs and Cre controls using DRIP-Sequencing. Integration with data from *Yang et al.* will reveal eRNA R-loops changes that correlation with enhancer dysregulation.

Aim 1B. Use RED-LasRR to determine how manipulating eRNA R-loops impacts enhancer formation and stability by assessing enhancer-promoter looping and target gene transcription

Described in a recent *Nature* publication in which our group participated, the RED-LasRR system is a CRISPR-dCas9 fused to RNaseH1 which is guided by sgRNA to an R-loop which it subsequently degrades[44]. Interestingly a nearly identical system was described one month earlier in *Science Advances* by *Li et al*. Of note, their system was demonstrated in primary MEFs[12], indicating the feasibility of ICE/Cre MEFs for this aim. First, I will use the same methods previously demonstrated[12,44] to transiently transfect ICE and Cre cells with RED (RNaseH1-eGFP-dCas9) or dRED (dRNaseH1-eGFP-dCas9) along with sgRNA. Of note, dRED contains catalytically dead RNaseH1 which is capable of binding R-loops, but not degrading them. It was previously shown that dRED (and the analogous construct by *Li et al*) actively protect R-loops from degradation and increase their stability[12,44]. To ensure that RED (or dRED) is active during the induction of epigenetic aging, transfection will take place 24 hours prior to treatment with 4-OHT. To ensure the validity and reproducibility of this design, significant optimization will be conducted in the manner described previously[44]. In particular, it will be necessary to first reproduce the previous results which showed successful targeting of the IGS28 and *ACTB* R-loops[44] and reprogramming-related R-loops[12]. Once the protocol is ready, I will begin testing whether the eRNA R-loops discovered in Aim 1A are necessary for enhancer stability. The design of this sub-aim is shown in **Figure 6**. Briefly, sgRNA target sequences for degrading the six highest confidence eRNA R-loops will be selected by evaluating the chromatin contact maps in ICE/Cre MEFs[5], selecting three sites per R-loop which are spatially proximal to it, and then designing (CRISPick, Broad Institute) and ordering the sgRNAs (Thermo). Two assays of enhancer dynamics will be performed: (1) 3C-qPCR to measure enhancer-promoter looping and (2) RT-qPCR to measure transcription of target genes. For each experiment, there will be 4 biological replicates per condition and hypothesis testing will proceed via multiple t-test comparisons with Benjamini Hochberg correction.

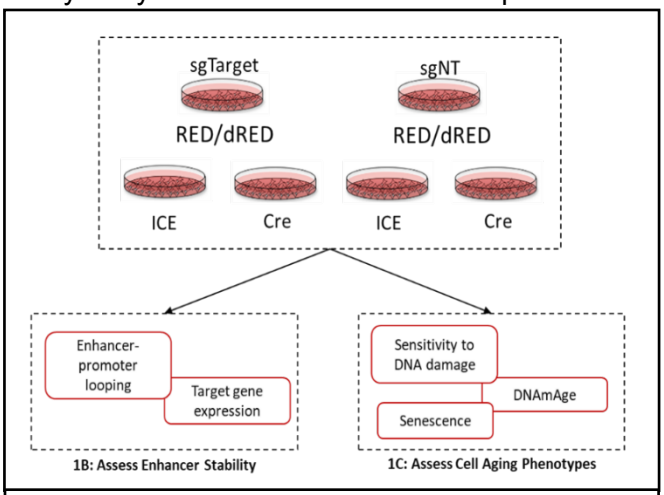


Figure 6: Aim 1B/C experimental strategy. R-loops discovered in Aim 1A will be degraded (RED) or protected (dRED) in ICE and Cre controls. For Aim 1B, enhancer stability will be assessed. For Aim 1C, cell aging phenotypes will be assessed.

3C-qPCR: Cells will be processed using the protocol previously described[56]: cells will be collected and disaggregated into a single cell suspension, then fixed and lysed to obtain nuclei. Chromatin will be digested using restriction enzymes and then ligated and purified. Then PCR will proceed using a OneStep kit and primers designed such that the 'constant' region is the enhancer or promoter. It is expected that protection of R-loops with dRED will prevent loss of enhancer-promoter looping in response to 4-OHT induction in ICE MEFs and that

disruption of R-loops with RED in Cre control MEFs will mimic the effects of 4-OHT induction in ICE MEFs by disrupting enhancer-promoter looping. Furthermore, it is anticipated that RED will exacerbate the effect of 4-OHT induction. **RT-qPCR**: Cells will be lysed, and RNA will be isolated using the RNeasy kit (Qiagen). RT-qPCR will then be performed with a OneStep kit to measure the levels of transcription at the enhancer target gene(s) and control genes like *GAPDH*. It is expected that protection of R-loops with dRED will prevent loss of target gene expression in response to 4-OHT induction in ICE MEFs and that disruption of R-loops with RED in Cre control MEFs will mimic the effects of 4-OHT induction in ICE MEFs by disrupting target gene expression. Furthermore, it is anticipated that RED will exacerbate the effect of 4-OHT induction.

Pitfalls, alternatives, and additional controls: As MEFs are notoriously difficult to transfect, it may be that case that lipofectamine or RNAiMax will be insufficient to ensure high-efficiency transfection. If this is the case, there are many MEF-optimized and CRISPR-optimized transfection kits/protocols (e.g., Altogen Biosystems) which may be tested. If necessary, it is also possible to use lentiviral transduction in order to generate stable cell lines. Furthermore, as MEFs can be quick to senesce, it may be necessary to establish a breeding colony of ICE/Cre mice (offered by Dr. Sinclair (*see letter*)). To ensure RED LasRR correctly degrades/protects R-loops, I will validate each target using DRIP-qPCR via the method previously described[47]. It is also possible that dRED will block normal co-factor interactions with R-loops, preventing enhancer stability. In that case, RED alone may be used to induce and exacerbate enhancer instability. It is also possible that no guide RNAs direct RED LasRR to successfully degrade an intended R-loop. This may occur because of the selection of a site which does not permit RED-LasRR sufficient access to the R-loop. Depending on the frequency of this occurrence, I will conduct a small screen within a range of potential sgRNA target sites around the R-loops of interest, providing valuable insight into how to appropriately use 3D contact maps in the selection of target sequences for RED-LasRR. However, it is also possible that no targets will prove effective in guiding RED LasRR to selectively degrade R-loops. This unlikely result would indicate that RED LasRR is not a suitable system for this project and the construct which has already been demonstrated by *Li et al* in MEF cells will be requested[12]. Additionally, a useful positive control for RED-LasRR (for RED, not dRED) will be to use siRNA targeting the eRNA in each case as previously demonstrated[6] or, alternatively, anti-sense oligonucleotides as was previously demonstrated[44].

Aim 1C. Use RED-LasRR to determine how manipulating eRNA R-loops impacts aging phenotypes
To validate the relationship between eRNA R-loops, enhancer stability, and epigenetic aging, the top three R-loops (that have effective sgRNAs) determined from Aims 1A/B will be targeted, and the impact on cell aging phenotypes, including sensitivity to DNA damaging agents, senescence, and accelerated methylation clock age[5], will be measured. Of note, this strategy mirrors the strategy successfully demonstrated in MEFs by *Li et al*[12]. The experimental groups are the same as Aim 1B. For these experiments, hypothesis testing will proceed using t-tests with multiple testing correction via Benjamini Hochberg. The design of this sub-aim is shown in **Figure 6**.

Sensitivity to DNA damaging agents: In the manner previously demonstrated,[5] cells will be treated with camptothecin (CPT) and assessed via immunofluorescence with quantification for the expression of γH2AX and 53BP1. It is expected that ICE cells will demonstrate significantly increased sensitivity to CPT, but that this phenotype will be rescued or exacerbated in ICE-dRED or ICE-RED cells, respectively. **Senescence**: In the manner previously demonstrated[5], cells will be stained for senescence-associated beta galactosidase (SABG) using a kit. The level of staining will be measured using bright field microscopy and quantified. Furthermore, qPCR will be used to evaluate the expression of senescence-associated secretory phenotype (SASP) factors such as IL-6 and Ccl2 as previously demonstrated[5]. It is expected that protecting or degrading the target R-loops will rescue or exacerbate, respectively, the senescence phenotype observed in ICE cells[5]. **Accelerated methylation clock age**: Finally, the effect of R-loops on epigenetic aging will be assessed by measuring methylation clock age. Reduced representation bisulfite sequencing (RRBS) will be conducted in the manner previously described.[5] It is expected that ICE cells will show accelerated methylation clock age and that protecting or degrading the target R-loops will rescue or exacerbate, respectively, this phenotype.

Pitfalls, alternatives, and additional controls: The main pitfall of this approach is the possibility that these R-loops occupy redundant roles and that targeting only three will not rescue or not fully rescue these phenotypes. This result is unlikely given that *Li et al* have already demonstrated the ability to target three R-loops and significantly impact a cellular phenotype in MEFs[12]. However, I can potentially address this issue by using network modeling to identify R-loop hubs from the results of Aim 1A. These hubs will be the most-likely candidates for controlling key enhancers and may be better targets for RED-LasRR. Furthermore, additional useful endpoints for epigenetic aging would be to assess p16/p21 expression and cell cytostatic impact.

SPECIFIC AIM 2: Determine the impact of R-loops on enhancer dysregulation during epigenetic aging. Aim 2 hypothesis**:** *STAG2 protects R-loops to support enhancer stability and preserve the youthful epigenome.*

Aim 2A. Test the effect of manipulating STAG2 and R-loops on aging phenotypes of ICE and Cre MEFs. To test the hypothesis that STAG2 protects eRNA R-loops to preserve enhancer stability and prevent epigenetic aging, I will manipulate STAG2 and RNaseH1 levels using siRNA and assess cell aging phenotypes and levels of epigenetic noise **Figure 7**. First, ICE/Cre MEFs will be transfected with siRNA targeting STAG2, RNaseH1, or scramble control (Thermo) using RNAiMax (Thermo). To ensure that STAG2 levels are decreased during the induction of epigenetic noise, transfection will be performed 24 hours prior to 4-OHT treatment. 96 hours following 4-OHT treatment, aging phenotypes will be assessed in the manner described in Aim 1C. It is expected that STAG2 depletion will exacerbate epigenetic aging in ICE MEFs and recapitulate the effects of ICE induction in Cre controls. However, because STAG2 protects R-loops from degradation by RNaseH1, it is also expected that simultaneous knock-down of RNaseH1 will partially rescue the aging phenotypes induced or exacerbated by the knockdown of STAG2.

Pitfalls, alternatives, and additional controls: Most pitfalls are similar to Aim 1C. To ensure STAG2 and RNaseH1 siRNA work as expected, we will quantify extent of depletion by western blotting, though partial depletion may be sufficient and less toxic for RNAseH1; alternatively, we can use constitutive inducible shRNA.

Aim 2B. Test the effect of manipulating STAG2 and R-loops on the development of epigenomic noise in ICE MEFs. To further uncover the interactions between STAG2, R-loops, and the aging epigenetic landscape, I will employ a second sequencing approach which is complementary to Aim 2A and designed to quantify the level of epigenetic noise induced by STAG2 knock down with or without parallel RNaseH1 knock down. The conditions for this sub aim are identical to Aim 2A. I will use H3K27ac and H3K27me3 ChIP-Seq to determine levels of epigenetic noise as demonstrated previously[5]. This sub aim is depicted in **Figure 7**. It is expected that enhancer stability is dependent on R-loops and that R-loops are protected by STAG2 from RNaseH1-mediated degradation. Consequently, I expect to observe that STAG2 siRNA exacerbates epigenetic noise in ICE cells and induces epigenetic noise in Cre controls. However, when co-transfected with RNaseH1 siRNA, the epigenetic noise phenotype should be partially rescued. It is also expected that RNaseH1 siRNA alone should provide some reduction in epigenetic noise in induced ICE MEFs.

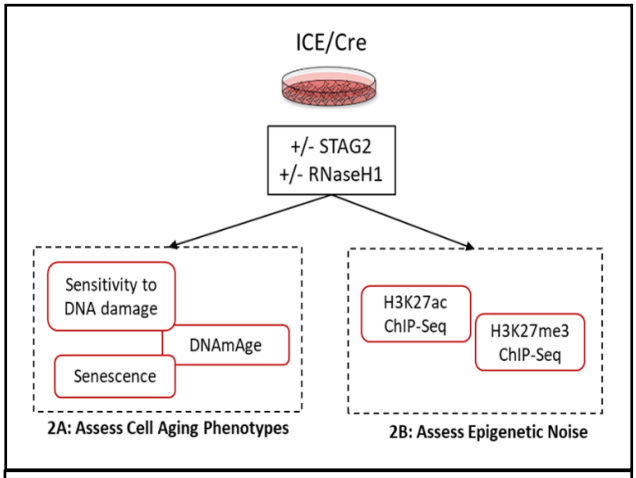


Figure 7: Aim 2A and 2B experimental strategy. STAG2 and RNaseH1 will be knocked down in ICE /Cre MEFs. For Aim 2A, cell aging phenotypes will be assessed. For Aim 2B: epigenetic noise will be assessed.

Pitfalls, alternatives, and additional controls: While the pitfalls of siRNA-mediated gene knock down were discussed in Aim 2A, it is worth noting the challenges inherent in ChIP-Sequencing. Fortunately, our collaborator, Jason Liu, who has extensive experience in epigenome profiling through ChIP-Sequencing, has agreed to assist in these experiments. Still, one pitfall of note is the possibility that the signal from aligned reads has an insufficient signal to noise ratio for peak calling. This will be addressed by including a genomic input control from which the background noise distribution can be effectively estimated. Another potential pitfall is that there will not be enough MEF cells to perform ChIP-Sequencing. Instead, CUT&RUN-Sequencing for H3K27ac and H3K27me3, which requires far fewer cells, can be used[57].

Aim 2C. Use ChIP-Sequencing to test whether STAG2 is differentially located to enhancers with epigenetic aging. Finally, to understand the reason for STAG2/R-loop interplay having an impact in enhancer stability with epigenetic aging, it is necessary to evaluate whether STAG2 is differentially re-located away from enhancers with induction of 4-OHT in ICE MEFs. ChIP-Seq for STAG2 will proceed using the methods previously demonstrated[17]. Differential binding of STAG2 will be calculated and differential peaks will be compared to sites of known enhancer dysregulation in ICE MEFs. It is expected that epigenetic aging causes a relocation of STAG2 away from enhancers, exposing their R-loops to degradation by RNaseH1, leading to enhancer collapse.

Pitfalls, alternatives, and additional controls: The pitfalls previously described regarding ChIP-Sequencing are relevant for this sub-aim as well. However, our laboratory has already successfully performed STAG2 ChIP-Sequencing in other unpublished experiments, so it is anticipated that the pitfalls for this aim will be minimal.

RESPECTIVE CONTRIBUTIONS

Over the last two years of being in Dr. Bishop's laboratory, we have discussed his interests and ideas with respect to physiological R-loop roles and enhancer biology. With this conceptual framework, I performed my own background research and preliminary analyses. Dr. Bishop encouraged me to apply for the F31. With his input, I designed the specific aims of this proposal. With the input of Dr. Bishop and Dr. Chen, I wrote the remainder of the training plan. Dr. Bishop and Dr. Chen have provided extensive feedback and edits on all aspects of this proposal.

I will be responsible for accomplishing the proposed research with the continued guidance of Dr. Bishop and Dr. Chen. Specifically, I will perform the experiments and analysis, coordinate any work done by institutional core facilities, and write the resulting manuscripts. Dr. Bishop will give regular feedback in weekly lab meetings and provide guidance throughout the week as I analyze results and troubleshoot any issues. Dr. Chen will also provide regular feedback and advice when needed regarding the bioinformatics components of the project. I will regularly give a formal presentation of my progress to both Dr. Bishop's lab and my dissertation supervising committee. The feedback from my lab members, dissertation supervising committee, and sponsors will be valuable in refining my dissertation and other manuscripts. In particular, I will write the initial draft of all manuscripts and then receive feedback from Dr. Bishop and Dr. Chen.

I am submitting this proposal with Dr. Bishop and Dr. Chen's approvals.

SELECTION OF SPONSOR AND INSTITUTION

Sponsor: My first rotation at UT Health San Antonio (UTHSA) was in Dr. Bishop's lab. His interest in combining wet- and dry-lab approaches to gain greater insights into complex biological systems was a trait I recognized as both uncommon and deeply aligned with my research interests. From the outset, Dr. Bishop was willing to provide me with many open-ended research questions to choose from and the promise of guidance should I ask for it, a management style which I find motivating. Furthermore, he encouraged me to learn new bench and computational techniques, providing an environment where I could receive high quality training in both skills sets from himself and his bioinformatics collaborator, Dr. Chen. The result was that, at the end of 6 weeks, I had grown my own cells, harvested RNA for sequencing, and then performed the differential expression analysis from scratch on the resulting dataset. In the nearly two years since, we have continued working together in this fashion, completing two original manuscripts and multiple manuscript reviews. In that time, Dr. Bishop has continually proven that he is chiefly concerned with his student's successful development as independent researchers. I have no doubt that Dr. Bishop is a perfect primary mentor for my fellowship training and for guiding me in the completion of my proposed project.

Co-sponsor: In my third rotation, I worked jointly with Drs. Bishop and Chen to benchmark splicing analysis algorithms for use in an upcoming project in the Bishop laboratory and for Dr. Chen's reference as he updated his standard analysis workflows. I found Dr. Chen to be an exceptionally knowledgeable and kind mentor who would regularly sacrifice his own time when I or others asked him for guidance. Though I did not officially join his lab, I have regularly sought Dr. Chen's guidance and advice on my various projects, and he is co-author with me and Dr. Bishop on one pre-print publication in BioRxiv already. After taking his genomic analysis course and co-planning a bioinformatics workshop together, I can confidently say that Dr. Chen is the perfect Co-sponsor to support this project because of his history of mentorship, long-time working relationship with Dr. Bishop, and vast expertise in computational biology.

Institution: The Barshop Institute at UTHSA was among my top choices for graduate study because of its storied history of excellence in aging biology research. In particular, the Barshop institute produced some of the biggest breakthroughs in aging research in recent years, from the studies of rapamycin's life-extending effects to the uncovering of transposon dysregulation by tau in Alzheimer's Disease. In particular, the Barshop Institute is the only aging research institute to house both a Claude D. Pepper and a Nathan Shock Center. It is also part of the NIA Interventions Testing Program and, with sufficient scientific rationale, students can successfully propose novel therapeutics for testing. Furthermore, I was attracted by the offer of a PhD discipline specifically tailored to the study of aging biology, one which provides coursework and seminars design to learn about cutting-edge topics in aging research, such as epigenetic clocks and novel senolytics. In the two years since I joined this program, I have found numerous opportunities for mentorship and training to support my future career goal of becoming an independent aging researcher, such as the opportunity to attend the Barshop annual conference in Bandera. Furthermore, though it was not an initial reason for me to apply to UTHSA, another benefit is the many bioinformatics experts, computational resources, and bioinformatic coursework at this institute. This includes multiple high-performance computing servers, several classes in bioinformatics and biostatistics, and mentorship from leading bioinformatics researchers like Dr. Chen. This has allowed me to effectively train as both a bench scientist and bioinformatician for my desired career as an independent aging biology researcher.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

Prior Training:

Responsible Conduct of Research (TSCI 5070).
I completed this course in Fall 2018. The course was directed by Drs. Kay Oyajobi and Kim Summers. The 2-hour/week course comprised didactic lectures from experts at UT Health San Antonio (UTHSA) regarding topics related to the responsible conduct of research. Attendance was required. Topics covered: 1. scientists as responsible members of society, 2. Policies for human and vertebrate research, 3. Collaborative research, 4. Conflicts of interest, 5. Data acquisition and laboratory tools, 6. Responsible authorship and publication, 7. Mentor/trainee responsibilities and relationships, 8. Peer review, 9. Research misconduct, 10. Informed consent, privacy regulations, good clinical practice, and special populations in clinical investigations. The course also involved a group project and presentation.

Experimental Design and Data Analysis (CSAT 5095).
I completed this course in spring 2019. The course was directed by Dr. Wouter Koek. The 2-hour/week course comprised didactic lectures, group assignments, homework, and student presentations. Topics covered included experimental design, controls, randomization, as well as statistical rigor and reporting.

Rigor and Reproducibility (CSAT 6005).
I completed this 4-hour/week course during a 4-week period in summer 2019. The course was directed by Dr. Wouter Koek. The course comprised didactic lectures, group assignments, and student presentations. The course involved multiple lectures from university faculty regarding scientific rigor, experimental design, authentication of key resources, statistics, data management and security, and reporting results. Additionally, the course discussed the importance of reproducibility in research.

Ongoing and Future Training:

Spotlight on Research Integrity.
This workshop meets throughout the year for 1-hour/month and is provided by the UTHSA Office of Postdoctoral Affairs. Workshops cover topics in the responsible conduct of research, including conflicts of interest, research compliance, record keeping, reproducibility, peer review, and ownership of data.

Biology of Aging Journal Club.
The Biology of Aging journal club is led by Dr. Karl Rodriguez for 1-hour/week. Students discuss recent articles which are impactful in the field of aging. Discussions often cover topics in the responsible conduct of research, including peer review, appropriate statistical approaches, and ethical portrayal of study results.

Laboratory Group Meetings.
The Bishop lab meets for 2-hours/week every week. Lab meetings often address topics in the responsible conduct of research such as authorship considerations, mentor/trainee relationships, collaboration, and peer review. In particular, Dr. Bishop regularly provides guidance regarding ethical considerations with respect to rigor and reproducibility of study results.

Institutional Online Training.
Online training in responsible conduct of research topics is regularly provided by UTHSA. Topics typically include conflict of interest, data management, sharing, and ownership, as well as research misconduct and animal welfare.

As I train for an independent career as an aging biologist, a field with many ethical considerations which have to be considered carefully, I am deeply committed to continuing my education in the responsible conduct of research.

SPONSOR AND CO-SPONSOR STATEMENTS

A. Research Support Available

Funding Source	Grant Number	PI	Grant Title	Project Period	1 Year Direct Costs
NIH	R01 CA241554	Bishop	Dysregulated transcription processes in Ewing sarcoma	05/2020-04/2025	$289,934
SU2C-CRUK	SU2C	Bishop	Targeting r-loop stability in Ewing sarcoma	10/2020-09/2022	$452,442
B+ Foundation	B+ FDTN	Bishop	Targeting RNA processing defects of Ewing sarcoma	01/2019-12/2020	$75,000
MERCK KGAA	MERCK	Bishop	Assessing the pathological accumulation of R-loops in cancer as an indication of sensitivity to RNA splicing inhibition	10/2019-10/2021	$213,482

Role of sponsor/co-sponsor: Dr. Bishop (**Sponsor**) has the main role as Henry Miller's mentor, both in guiding his overall research project, day-to-day supervision, helping identify courses and meetings to attend, developing his career plan and generally making sure that he has all the data, equipment, facilities, reagents and collaborators needed to succeed. As Henry's project is integrated with multiple other projects in Dr. Bishop's lab, Dr. Bishop will also be responsible for the overall integration of Henry's work so as to take advantage of all the opportunities and expertise that is present in the lab to facilitate Henry's project and education. Dr. Chen (**Co-Sponsor**) provides both computational and bioinformatics expertise. Drs. Chen and Bishop have a long standing successful working relationship that has resulted in multiple grants, papers and co-training of fellows. Dr. Chen's expertise complements that of Dr. Bishop and is key to the bioinformatics and computational aspects of Henry's training. Dr. Chen is not only a world renown computational scientist, but also the director of our computational core and provides access to computer nodes needed for Henry's project. Dr. Bishop and Henry are in daily contact and Henry actively participates in weekly lab meetings. Dr. Chen interacts with Henry as needed (usually weekly) and Henry meets with Drs. Bishop and Chen once a month.

B. Sponsor's/Co-Sponsors Previous Fellows/ Trainees

Dr. Bishop is a tenured Associate Professor in the Department of Cell Systems and Anatomy at UTH-SA and is located in the GCCRI. He has graduated six PhD students, two MS students, and five postdoctoral scholars since his independent career began in 2005. Dr. Chen is a tenured Professor in the Department of Population Health Sciences at UTH-SA and is located in the GCCRI. He has graduated 5 PhD students and mentored 7 postdoctoral scholars since his independent career began in 2008. Of note, my prior PhD student, Aparna Gorthi, joined Dr. Chen's group as a postdoctoral fellow, before obtaining a fellowship where she is under both mine and Dr. Chen's supervision.

Trainee	Degree Obtained	Training Period	Research Topic	Current Position
Aparna Gorthi	PhD	08/2011-08/2015 and then 08/2018-present as a postdoc	Chemosensitivity of Ewing sarcoma. She obtained several fellowships and awards over her tenure. She published 10 papers (1 first author in Nature) while a PhD student in my lab and then 4 more when she returned to work in my lab (2 co-author in Nature), for 17 papers during her training, and still has several manuscripts to be submitted.	Postdoctoral Fellow, UTHSCSA, San Antonio, TX (about to be promoted to Instructor level)
Bijal Karia	PhD	08/2006-06/2013	p53 suppression of homologous recombination and tumorigenesis. She obtained a DoD fellowship, published 5 papers (1 first author) and 1 book chapter while in my lab. Another manuscript (first author) is ready for submission.	Scientist, bioAffinity, San Antonio, TX

Alfeu Zanotto-Filho	Postdoctoral Fellow	01/2013-09/2014	Investigating alkylation survival in breast cancer, obtained DoD fellowship support, published 3 first author papers while in the lab and has another under preparation	Associate Professor (tenured), Universidade Federal de Santa Catarina, Brazil
Amy Wiles	Postdoctoral Fellow	09/2005-12/2009	Developing tools for comparative genomic screening and analysis. She published 3 papers (2 as first author) and one book chapter while in my lab.	Associate Professor (tenured), Mercier University, GA
Dashnamoorthy Ravi	Postdoctoral Fellow	06/2005-12/2007	Using *Drosophila* RNAi screening to improve our understanding of DNA damage survival. He published 9 papers (4 as first author) while in my lab.	Associate Professor (research track), Rutgers Cancer Institute, NJ

C. Training Plan, Environment, Research Facilities

Training Plan

The training plan developed for Henry was design specifically to both challenge him and train him for a successful career in the field of systems biology as applied to aging. Since Henry joined my laboratory, we outlined a plan to develop several aspects of his training, based on the expectations of the school, myself and his own aspirations and interests. It is with a mind to his interests in bioinformatics that we included Dr. Yidong Chen (long term collaborator) as a co-sponsor for Henry. Henry and I have established six main goals, to be achieved during his training, so that he can become a successful independent researcher: (1) to build a strong bench research skillset, (2) to strengthen his computational expertise, (3) to develop his communication and presentation skills, (4) to learn to conduct independent research, (5) to gain experience with teaching and mentoring students, (6) to gain experience managing student researchers. We plan to achieve these goals by addressing the following aspects:

(1) Build a strong bench research skillset & (2) Strengthen computational expertise: Henry's project focuses on expanding our understanding of the biology of physiological R-loops and their potential dysregulation in aging. To-date most work with R-loops has been with respect to their association with transcription, mainly transcription regulation, and then how the occurrence of R-loops at the wrong time/location can interfere with replication and cause genome instability. Our lab has been investigating these phenomena for the last several years and has expertise in the genomic sequencing of R-loops (DRIP-Seq). As our expertise is in molecular and cell biology, particularly DNA repair, we worked with **Dr. Yidong Chen**, Director of the Computational Biology & Bioinformatics Core (and co-Sponsor for Henry's application), to develop much of the specialized bioinformatics necessary for these analyses. Importantly, the field of R-loop mapping and analysis is underdeveloped with many unanswered questions and a lack of tools for their study. As such, Henry will be taking advantage of both the expertise within my lab to produce high quality data and that of Dr. Chen to perform the analyses. Of note, our University is well known for its Biology of Aging research, through the Sam and Ann Barshop Institute for Longevity and Aging Studies, of which I am a longstanding member. Though much of my research involves cancer, this is mainly around DNA repair and DNA repair syndromes that often display both cancer predisposition and segmental progeroid phenotypes. Henry is part of our Biology of Aging PhD program and he takes advantage of the vast aging research expertise which it offers. I believe this places Henry in an ideal situation to evaluate R-loops with the methods and techniques well established in both mine and Yidong's labs, but in an aging context. Henry's research will require several methods including bioinformatics and cell biology/genomics techniques. Henry has already established himself with bioinformatics analyses, being named on multiple papers for contributing those types of analyses in other people's projects, while also publishing a first author paper which largely used bioinformatics in addition to wet lab techniques, all

while having a second under review and a third in preparation from bioinformatics software he developed. He is also working on the bench to establish the lab methods he needs for his project, which will include DRIP-Seq, ChIP-Seq, qPCRs, and western blotting. Henry will enhance his molecular techniques such as fluorescence microscopy, molecular cloning, and protein-DNA interaction analysis. Henry will be trained in all these techniques which are routinely performed in my laboratory or be provided the necessary guidance from our collaborator, Jason Liu, an expert in ChIP-sequencing and enhancer biology.

(3) Develop his communication and presentation skills & (4) learn to conduct independent research: The most common types of scientific communications are publication in a peer reviewed journal and scientific talks at a national or international symposium. Accordingly, Henry's training will develop both aspects.

First, I will ensure that Henry becomes familiar with the format of a research manuscript. In fact, to ensure their development of independent research skills, I insist that all my students and postdocs write the first draft of their manuscripts and Henry will be no exception. As Henry is highly organized, a strong writer and has already demonstrated the ability to put together and publish a manuscript from scratch in my lab, I do not see any concerns on this front for him. Furthermore, to support Henry's development as an independent researcher, I will insist on his applying for funding opportunities such as this F31 and, later-on, predoctoral to post-doctoral transition funding mechanisms. I will personally guide him in his developing grantsmanship, but he will also have the opportunity to seek expert advice through his participation in on-campus grant writing workshops like "F-Troop". By strengthening his grant writing abilities now, Henry will progress along the path to achieving independent funding. As he has now already applied for and received one such fellowship (Greehey Graduate Fellowship), I am confident on this score as well. Henry will also learn to conduct independent research as he completes the research aims in this proposal.

Secondly, Henry will continue to participate in the Biology of Aging and Bioinformatics Journal Clubs and Barshop Institute Seminar Series. Additional opportunities to improve his scientific articulation skills include three annual retreats: GCCRI, CS&A and Barshop Institute. In this same avenue, in order to provide him with exposure to the general scientific community, I encourage Henry to participate in relevant national and international conferences including the American Aging Association Annual Meeting and Gordon Conference on Aging, as well as relevant Workshops such as the Machine Learning for Single Cell Analysis workshop. Several of my students and postdocs have been given opportunities to present at national meetings in their own right or in my stead (Keystone, Gordon, CSHL). Given that Henry is able to present his results to our collaborators, articulating his point clearly and succinctly, I again see little issue in developing these skills.

Additionally, I serve as an *ad hoc* reviewer for multiple journals. Henry will be encouraged to participate in the peer review process when appropriate and coached on how to critically evaluate research activities of others. Where appropriate, he will be acknowledged for these endeavors.

(5) Gain experience with teaching and mentoring students & (6) gain experience managing student researchers: In order to develop his skills as a mentor and principal investigator when he establishes his own laboratory, Henry will have the opportunity to mentor other, more junior, students. He is already mentoring and managing a team of several undergraduate and master's students as part of several short projects which they are working on together. Henry is also interested in becoming a lecturing professor in the future. He is already participating in opportunities to plan and implement new bioinformatics curricula with his participation in the bioinformatics curricula committee at UTH-SA and R workshop which he and Dr. Chen co-planned. This semester, these efforts continued to expand as Henry planned and led an ambitious 14-week R-programming and RNA-Seq analysis workshop which was attended by dozens of students. He has also designed a bioinformatics graduate certificate which has gained preliminary support from our graduate school. Henry also plans to enroll in UTH-SA courses designed to give hands-on instruction in planning a curriculum and lecturing.

Meetings with Sponsor/co-Sponsor: My laboratory has weekly meetings that alternate between informal discussions by everyone and a formal presentation by each individual. During the informal meetings, all members (postdocs, graduate students and research staff) discuss work updates, recent literature in the field, manuscript organization and future projects. These laboratory meetings also provide a great environment to address problems faced in experiments and constructive critiquing of the science and methodology. Henry actively participates in these group discussions and also formally presents his work at regular intervals to improve his presentation and scientific communication skills and receive feedback on his progress. One-on-one meetings take place as needed, at least twice a month. During these meetings, students and postdocs discuss their progress and receive feedback on the design of future experiments. In addition, we discuss abstract preparations, extramural funding applications as well as manuscript preparations. In addition, I have

an open-door (or open invitation to contact by Zoom) policy and strongly encourage informal discussions on a daily basis with students about their experiments and results. In addition, Dr. Chen is equally available. In addition, Henry participates in Dr. Chen's bioinformatics journal club and deep learning journal club bi-weekly. Moreover, Drs. Chen and Bishop often have joint meetings including students and postdocs to allow a free flow of ideas and setting of deliverables.

Coursework: Henry successfully completed all core courses and 2 of 6 elective credits required by the Biology of Aging Program. The additional required credits will be completed by Summer 2022. Additionally, Henry is required to take at least 6 credits of the "Dissertation" course over at least two semesters in order to graduate. Finally, this Fall Henry will have his dissertation proposal. I expect that he will pass this milestone to become a PhD candidate without any issue. Additionally, in order to provide Henry with additional bioinformatics training, he will complete the "NGS Data Analysis" course, and, because Henry has expressed a strong interest in becoming a professor, he will also take "Introduction to Science of Teaching" and "Supervised Teaching", courses designed to provide hands-on training in course design and practice lecturing.

Planned coursework during F31			Y1	Y2	Y3
Course ID	Title	2021	2022	2023	2024
IBMS 6097	Research	X	X	X	
IBMS 7099	Dissertation		X	X	X
INTD 6062	NGS Data Analysis	X			
INTD 6011	Introduction to Science of Teaching		X		
CSAT 6071	Supervised Teaching			X	X

Responsible Conduct of Research: I have a commitment to train Henry in an integral manner, and I take very seriously the training and the completion of the endeavors regarding responsible conduct of research. Henry has completed a course on Responsible Conduct of Research (TSCI 5070), which is an in-depth course that includes conflict of interest, mentor/mentee responsibilities, peer review, research misconducts, whistle blowing, strategies for self-assessment and validation of objectivity, etc. Henry has also taken two other courses, Experimental Design and Data Analysis (CSAT 5095) and Rigor and Reproducibility (CSAT 6005), which cover topics related to proper experimental design, data management, storage, and privacy, as well as robust statistical approaches, authentication of key resources, reproducibility, reporting, controls and randomization. In addition, Henry will participate in Spotlight on Research Integrity, a monthly workshop which provides training on RCR topics such as peer review and conflicts of interest. Henry will also continue to participate in institutional online trainings for RCR topics like research misconduct. Furthermore, I actively discuss ethical issues relevant to research, discussing the peer review process, and responsible data collection and interpretation during laboratory meetings and one-on-one meetings,

Environment

Henry will be exposed to a highly interactive and scientifically challenging environment. The Department of Cell Systems and Anatomy, which is my academic home, is known as a research-oriented, interdisciplinary Department and is well known for its excellence and productivity. In addition, I am an active member of the Barshop Institute. Henry has the opportunity to interact and collaborate with many faculty members, from very diverse areas of expertise. My (and Dr. Chen's) laboratory is located at the Greehey Children's Cancer Research Institute (GCCRI) within UTH-SA, across the street from the Barshop Institute. The main objective of the GCCRI is the advancement of cancer research directly related to children's health. The faculty at GCCRI come from a variety of backgrounds and have strengths in a broad range of research and technical expertise. Research areas include stem cells, miRNA, lncRNA, DNA repair, RNA binding, mouse models, preclinical PDX models, X-ray crystallography, NMR and bioinformatics. This compilation of different expertise and the open lab structure of our working space were designed on purpose, to develop a community of researchers who could interact and synergize to tackle cancer research with novel ideas. This environment offers Henry the opportunity to exchange ideas, strengthen collaborations and benefit from access to several resources such as shared equipment and assistance from administrative and support staff.

I am also a member of the NCI designated cancer center at UTH-SA (the Mays Cancer Center) and hold a programmatic membership in the Department of Molecular Medicine. Through my location and memberships,

Henry has access to every part of UTH-SA and all the resources therein (including the Genomics, Mass Spectrometry, Next Generation Sequencing (located at GCCRI) and Bioinformatics (located at GCCRI) Cores). Within my laboratory I have everything needed to perform cell culture, molecular biology and mouse work. The GCCRI has invested in substantial capital equipment such as a both HiSeq and 10X Single cell sequencer facilities, not to mention a robust IT infrastructure. This is a very vibrant, interactive and collaborative group that will provide Henry with significant guidance. Beyond this, I have an extensive collaborative relationship with **Dr. Chen** who provides the bioinformatics and computational aspects of this proposal and is highly supportive of Henry and his project. Knowing the direction of Henry's project, I have introduced him to another of my collaborators, Jason Liu, who is an expert in enhancer biology and discovered the presence of eRNA and MegaTrans factors at enhancers and Hong Wang, who is an expert in biophysical approaches and with whom Henry is already an author on a recent publication relevant to his proposed aims. Finally, it should be noted that Henry's "environment" extends well beyond UTH-SA, as Henry has not only become involved in multiple collaborative projects that were running in my lab, but he also cultivated new collaborations necessary for his project that I have happily supported. I am confident that with these resources, infrastructure and collaborations Henry's proposed research project will be completed successfully.

Research Facilities

Laboratory: The laboratory has all the equipment for molecular biology and tissue culture. This includes 975 square feet of bench space (3.200.F1 and 3.200.H1), an adjacent tissue culture suite of 225 square feet (4.200.02A) and shared equipment rooms for larger equipment and liquid nitrogen storage. The tissue culture suite has 6 incubators (four with N2 lines, one housing an Essen IncuCyte), two biological safety hoods, a combination refridgerator/freezer and a refridgerated tabletop centrifuge. We have 1 large refridgerator, 2 undercounter refridgerators, three -20 freezers and two -80 freezers.

Computer: All personnel have Apple or Dell desktop computers with all needed software, network to color printers and a flatbed scanner. In addition, computers for equipment and microscopes are provided. For large data analysis and image manipulation we have access to a ~10 Linux servers (with ~500GB RAM and up to 192 cores per server) and most advanced GPUs managed by Dr. Chen's group. The GCCRI provides webpage support and server facilities with tape backup, oracle and SQL databases, citrix metaframe and secure FTP sites. Computers are loaded with all the necessary research tools including Endnote, GraphPad Prism, OpenLab, Gene Construction Kit, Acrobat suite and a license for Ingenuity Pathway Analysis.

Office: The office of Dr. Bishop is 128 square feet (Room 3.100.16) with additional file storage adjacent. Desk space, file storage and a computer are provided to all postdocs and PhD students (3.100.A1-A2, A4-A5).

Other: Shared administrative services are provided. We have access to seminar rooms (3.200.D2 and 3.200.E1) with projection and video conferencing capability. Centralized glass washing and microtip loading/autoclaving is provided by the GCCRI. UTH-SA provides an extensive electronic library.

D. Number of Fellows/Trainees to be supervised during the Fellowship

Trainee	Seeking Degree	Expected Period	Research Topic	Current Funding
Liesl Lawrence	PhD	01/2016-06/2021	Transcription, R-loops and RNA Splicing in Ewing Sarcoma	CDMRP PRCRP Horizon Fellowship CA181177
Kevin Kanda	PhD	01/2019-06/2024	*Ewing Sarcoma relies on Endogenous Cysteine and Glutamine for Antioxidant Response*	Mays Cancer Center Fellowship
Pramiti Mukhopadhyay	PhD	01/2019-06/2024	Epigenetic changes in Ewing sarcoma	CPRIT Training Grant RP170345 Fellowship
Aparna Gorthi (co-mentor with Yidong Chen)	Postdoctoral Fellow	08/2018-07/2021	*Identifying Modifiers of PARP1 Inhibitor Sensitivity in BRCA-like Tumors*	AACR-AstraZeneca START fellowship

Nick Bassani	Postdoctoral Fellow	07/2018-06/2022	*Delineating and targeting mechanisms of Ewing sarcoma metastasis*	MERCK and B+ Foundation
Momin Rahman	Postdoctoral Fellow	03/2021-02/2024	Targeting SRSF2, SF3B1 and DHX9 in Ewing sarcoma	SU2C-CRUK
Valerie Caro	Post Baccalaureate	07/2020 - 05/2021	Targeting SRSF2, SF3B1 and DHX9 in Ewing sarcoma	UTH-SA PREP program

E. Applicant's Qualifications and Potential for a Research Career

With great enthusiasm I strongly recommend Henry Miller for the Ruth L. Kirschstein National Research Service Awards for an Individual Predoctoral Fellowship. Henry is an extremely motivated, energetic and capable 3rd year IBMS student. Henry sought me out to rotate in our lab because, though we are known for molecular biology, we also tend to use large datasets and state-of-the-art genomics methods, often developing our own bioinformatics tools or interfacing with Dr. Chen's bioinformatics group. Outright, upon rotating in my lab, I thought Henry would not only be a good fit with my group, but he would thrive in the environment. His self-starter attitude, general curiosity and strong work ethic has impressed me to no end. The quality of his work is outstanding and has even impressed both my bioinformatics colleagues and several of my collaborators (two of whom are writing letters of recommendation). Not only has Henry augmented our bioinformatics capability, but in realizing some of the questions we are asking he started to build tools to facilitate this work, one which is under review for publication and another which is in preparation. He has also used his insights in bioinformatics and R-loop biology to publish a first author paper, is a co-author in a NAR paper and in a Cell Reports paper, and he has become integral in several collaborative projects, including Drs. Chedin (R-loop expert) and Venkitaraman (BRCA2 expert). Just to remind the reviewers, Henry is only in his third year of grad school. In addition to his bioinformatics work, I also encourage Henry to pursue molecular and cell biology bench work. By excelling in both bench and bioinformatics skills, he will have no trouble in succeeding as a researcher. In just the last few months I have seen him grown in understanding and technical capability. I have no doubt he will continue to grow as his project develops. In the meantime, he has become integral to most of the ongoing projects in the lab. These experiences have not slowed him in his own research project, but rather provided various insights into R-loops that he is leveraging for his own pursuits. Importantly, Henry reads voraciously around the topics he is interested in, and he came up with the premise for his work, based on ongoing R-loop biology in my lab and his interest in aging and the idea of epigenetic aging.

Henry did his undergraduate studies at Christopher Newport University, with a double major in neuroscience and philosophy. He went on to obtain a masters at the University of Buffalo while also getting involved in two different start-up companies. In 2018 Henry joined our graduate program here at UTH-SA. If you follow his grades through his various university experiences, you will see that he has improved and grown as he has found his real interest. He currently holds a 4.0 GPA and I can say from teaching him in a couple of formal courses (IBMS5000 and Biology of Aging Core course) that he is bright, proactive and interactive. He asks very insightful questions and has a very clear mind, approaching problems logically and systematically. I was very impressed with not only his project presentation exercise for the Biology of Aging course, but the questions and interest he showed in the other students' presentations. I think it is for these reasons that I see other students gravitate to him. I have absolutely no doubt that he will go far in his research career.

Motivation and interest are the two major criteria I use when deciding whether to accept a rotating student into my research group. Obviously from my description above, Henry is highly motivated and very interested in all aspects of the work of my lab. He always asks questions on others projects and offers to help. I think the only difficulty that Henry will encounter through his graduate studies will be keeping focus to be able to complete his main project in a timely manner. However, with his organizational skills, I believe Henry can do this too.

Overall, as I have tried to iterate several times through this letter, I believe that Henry is an outstanding PhD student. I believe he has found his calling in a mix of computational bioinformatics and molecular biology; this is exactly the type of work that his project involves. Henry's growth through his educational experiences, his arrival into this graduate program demonstrates his progression to find the right direction to pursue. In conclusion, I strongly recommend Henry for this award as it would aid him immensely in the pursuit and accomplishing his professional goals to become an independent researcher exploring the biology of aging.

GLENN FOUNDATION
FOR MEDICAL RESEARCH

BLAVATNIK INSTITUTE
HARVARD MEDICAL SCHOOL

David A. Sinclair, Ph.D., A.O.
Professor and Co-Director
Glenn Center for the Biology of Aging Research
Blavatnik Institute, Harvard Medical School
Department of Genetics
77 Ave. Louis Pasteur, Boston MA 02115
(617) 432-3931
david_sinclair@hms.harvard.edu

December 1, 2020

Henry Miller, Bishop Laboratory
UT Health San Antonio

RE: Fellowship: NRSA F31 (FOA: PA-21-051)

Dear Henry,

I am writing to assure you that, when requested, our laboratory will supply you with the ICE and Cre control MEFs and mouse lines. We have already offered to supply you with both the mouse lines and cell lines, though you have only requested and received the cell lines to date. We recognize that these are key reagents in your proposed research project and are happy to send them to you when requested.

Sincerely,

David A. Sinclair

North Carolina State University is a land-Grant university and a constituent institution Of The University of North Carolina

Department of Physics
Campus Box 8202
Raleigh NC 27695-8202

NC STATE UNIVERSITY

919.515.2521 (phone)
919.515.6538 (FAX)

Henry Miller, Bishop Lab,
GCCRI December 6, 2020
RE: Fellowship: NRSA F31 (FOA: PA-21-051)

Dear Henry,
I am writing to express my enthusiasm for your proposed collaboration to explore the role of R-loops and STAG2 in regulating enhancer activity within the aging epigenome. The aims which you discussed are central to your NIH F31 proposal entitled “The dynamics and impact of R-loops in epigenetic stability and aging”.

I am currently an Associate Professor in the department of Physics at North Carolina State University. The research in my group involves the application of a broad range of biochemical and biophysical assays, including single-molecule atomic force microscopy (AFM) and fluorescence imaging, to study the conformation and dynamics of proteins involved in telomere maintenance, DNA repair, and epigenetic regulation. I have published more than 40 research papers in leading scientific journals, including Molecular Cell, Nature Communications, PNAS, and Nucleic Acids Research. My research has been funded by National Institutes of Health since 2008 (one K99/R00, two R01s).

As you know, our laboratories recently collaborated to study the interaction of R-loops and STAG2, the results of which were published in an article in *Nucleic Acids Research* this year on which we were both authors. After this recent publication of our findings, we continue our collaboration. Most recently, we discovered that STAG2 might protect R-loops from degradation by RNaseH1, a finding which supports the hypothesis on which your aims are founded.
It is because of my expertise in biophysical methods and our shared collaboration that you have asked me to lend my support for any biophysical experiments which may be of interest in the pursuit of your fellowship proposal aims. While your proposal does not currently list any such experiments explicitly, it is likely that positive results will lead to an interest in pursuing biophysical validation through *in vitro* assays such as atomic force microscopy, techniques which our laboratory has a great deal of experience with.

In short, we are enthusiastic for your proposal and are happy to contribute our technical capabilities in biophysical methods to support your proposed research aims.

Sincerely,

Hong Wang

_____

Hong Wang

Associate Professor
Physics Department
North Carolina State University
2401 Stinson Drive, Riddick 258E
Raleigh, NC 27695-8202
Phone: 919-513-72 email:hong_wang@ncsu.edu

Henry Miller, Bishop Lab, GCCRI

December 1, 2020

RE: Fellowship: NRSA F31 (FOA: PA-21-051)

Dear Henry,

I am writing to express my enthusiasm for your proposed collaboration to explore the role of R-loops in regulating enhancer activity and within the aging epigenome. The experiments which you discussed are central to your NIH F31 proposal entitled "The dynamics and impact of R-loops in epigenetic stability and aging".

To introduce my background: I am currently an Assistant Professor in the department of Molecular Medicine at UTHSCSA. As you know, I have been studying the enhancer complex assembly mechanisms for almost ten years, first as a post-doctoral fellow in Dr. Michael Rosenfeld's lab at UCSD (Liu et al., *Cell* 2014. **159**: 358-373) and now as an independent principle investigator at UTHSCSA (Zhu et al., *Mol. Cell* 2019. **75**: 791-806). In a recently published study, we used ChIP-sequencing to demonstrate that endocrine therapy resistance induces genome-wide enhancer reprogramming and promotes basal/mesenchymal cell states in breast cancer (Bi et al., *Nat. Cell Biol.* 2020. **22**: 701-715). As you have observed, our lab regularly performs ChIP-sequencing experiments, and we have a great deal of expertise in enhancer biology. You expressed that these are the reasons you hope we may collaborate on key experiments in your proposed research project, a request I will gladly fulfil.

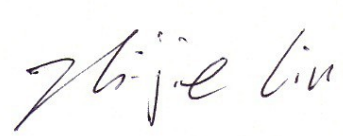
In your second aim, you propose to use ChIP-sequencing to profile epigenetic noise and enhancer dysregulation in response to epigenetic aging induction with or without disruption of STAG2 and R-loops. For this experiment, you propose to examine histone markers H3K27ac and H3K27me3. You also propose to study differential STAG2 binding with and without epigenetic aging induction. I am enthusiastic to provide you with training and assistance for each of these experiments. We have extensive experience in ChIPing and would be happy to train you so you can successfully perform those sequencing experiments yourself. We also have experience in bioinformatics, particularly with respect to analyzing epigenomic changes from the integrative analysis of multiple sequencing datasets. As such, we will be glad to provide you with training and assistance in the analysis and visualization of your sequencing data sets.

Our laboratories are already collaborating on multiple projects and we have previously trained several students in your research group in ChIP-sequencing. It is in this continued spirit of collaboration that we

DEPARTMENT OF MOLECULAR MEDICINE / **Institute of Biotechnology** / 8403 Floyd Curl Drive /Mail Code 8257
South Texas Research Facility / San Antonio, Texas 78229-3900
www.uthscsa.edu / www.molecularmedicine.uthscsa.edu

indicate our enthusiasm for your proposal and our willingness to contribute training in ChIP-sequencing and bioinformatics for the successful completion of your aims as well as your development as a PhD student and future investigator.

Sincerely,



Zhijie (Jason) Liu, PhD
Assistant Professor
CPRIT Scholar in Cancer Research
V Foundation Scholar in Cancer Research

UT Health San Antonio
Graduate School of Biomedical Sciences

December 5, 2020

Institutional Letter of Support

As Senior Associate Dean of Admissions and Student Affairs in the Graduate School of Biomedical Sciences (GSBS), I am delighted to provide the following description of our Integrated Biomedical Science (IBMS) Graduate training program, and our commitment to Mr. Henry Elvis Leroy Miller. The goal of the IBMS training program at the UT Health San Antonio (UT Health) is to prepare the next generation of independent scientists by providing an integrated, cohesive, educational experience. Graduates of this program have successfully integrated into the scientific work force evidenced by their productive careers as leaders in academe, government and/or the pharmaceutical and biotechnology industries. We fully expect that our current students, including Mr. Miller, will follow in this time-honored tradition.

IBMS Program and Research Disciplines

The GSBS conducts cutting edge, basic and translational science research directed at an improved understanding of human health and disease. The research activities of over 300 faculty members in the IBMS are diverse and range from very basic to clinically oriented research. IBMS thematic disciplines are aligned with the major research foci of the faculty and include:

1) Biology of Aging
2) Cancer Biology
3) Cell Biology, Genetics and Molecular Medicine
4) Molecular Immunology and Microbiology
5) Biochemical Mechanisms of Medicine
6) Neuroscience
7) Physiology and Pharmacology

Individual discipline leadership is comprised of two senior faculty members who provide advice, support and guidance to students on course selection and research mentorship. Mr. Miller is currently being trained in the Biology of Aging (BA) discipline which is housed in the Barshop Institute. The Barshop institute is the only institute to house both a Nathan Shock Center and a Claude D. Pepper Center for Biology of Aging research. They are also part of the NIA's Interventions Testing Program. With these exceptional resources, Barshop Institute researchers have made tremendous advancements in our understanding of the biology of aging. Faculty in the discipline possess a broad range of expertise and provide training in areas of aging, neurology, physiology, comparative biology, , optical imaging, molecular genetics of human disease, stem cell, use of genetic animal models, proteomics, and reproductive and developmental biology among others. Emphasis of the program of study and research is on flexibility in order to provide a tailored experience to meet the individual needs and interests of the students. A highly interactive community of faculty, post-doctoral fellows, laboratory staff and students work well with other departments and disciplines to create a challenging, stimulating and supportive environment within which our students develop into successful scientists.

Curricula

Students enrolled in the IBMS enter as "undifferentiated"; that is, they have not selected a specific discipline. In their first year, students take an interdisciplinary course, Fundamentals of Biomedical Sciences, which includes essential information in the basic biological sciences. In the second semester, they select a specific IBMS discipline and a dissertation-supervising professor for further training through course work and research. Responsible Conduct in Research and Rigor and Reproducibility are mandatory for all GSBS students. The curriculum is sufficiently interdisciplinary and students in a particular discipline are encouraged to take courses offered by other disciplines. During the second year, students continue to take discipline-specific electives and journal clubs, participate in seminars, and engage in research. Major milestones include the

UT Health San Antonio
Graduate School of Biomedical Sciences

advancement to PhD candidacy exam at the end of the second year and the formal approval of a dissertation supervising committee shortly thereafter. From admission to candidacy through granting of the degree, years 3-4 and 5, if necessary, each student is responsible for submitting and defending the dissertation research proposal, rigorously working in the laboratory collecting data, presenting research findings at local, national and international meetings, publishing at least one peer-reviewed manuscript, completing the program of study and successfully defending the dissertation research work. The time to degree for students in the IBMS averages 5.5 years.

Student's Progress

Henry matriculated into the integrated graduate program in Fall 2018 and joined the laboratory of Dr. Alexander Bishop, an Associate Professor in the Department of Cell Systems and Anatomy in December 2018. Henry successfully passed his qualifying exam in May 2020, will complete his dissertation proposal in Fall 2020 and is currently in good academic standing with a 4.0 GPA (49 semester credit hours). His research project entitled "The dynamics and impact of R-loops in epigenetic stability and aging" explores the use of bioinformatics to study R-loops and their influence on epigenetic stability as a molecular mechanism of aging. He is making excellent progress and is expected to complete his doctoral degree no later than August 2024.

Governance of the IBMS

The Dean of the GSBS is the administrative head of the graduate programs and serves as the Chair of the Graduate Faculty Council (GFC). The Committee on Graduate Studies (COGS) of the program is composed of members of the graduate faculty and is responsible for monitoring students' academic progress in didactic and research activities, attesting eligibility for admission to candidacy for a degree, and verifying to the GFC that the student fulfilled all requirements for the awarding of the degree. The Chair of the COGS is the administrative head of each program and serves as the voting representative for the program on the GFC and the liaison officer between the COGS and the Graduate Dean's office on all matters pertaining to applicant and student affairs. The GFC has the responsibility to establish and maintain policies and regulations on matters of graduate education common to all programs administered by the GSBS. Standards of student professional conduct, grading systems, graduate program review and criteria for dissertation research, its supervision, and its defense are all the purview of the GFC.

The Graduate School of Biomedical Sciences strongly support Mr. Miller's application for this fellowship and guarantee that he will have full access to the programs and resources outlined above. We believe that our faculty strives to achieve outstanding success in their educational mission of preparing professional young scientists who can function and perform with excellence in the academic, industrial, and/or government sectors. There is a diversity of talent, but a unified purpose in teaching and mentoring graduate students in an array of interdisciplinary fields of study and research. In the context of this exciting research environment, Mr. Miller is provided the resources and mentoring to enhance his training during his quest for becoming an exceptional independent investigator in the field of Biology of Aging.

Nicquet M.J. Blake, PhD
Senior Associate Dean
Admissions and Student Affairs

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	❍ Yes ● No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	
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
Other Requested Information	

Resource sharing plan

Data Sharing Plan: The proposed research does not involve a budget of $500,000 or more in direct costs. All results generated by this project will be shared with the scientific community by publication in peer-reviewed journals, with full acknowledgement of NIH support.

Mr. Miller, with the help of Dr. Chen, will deploy mechanisms for data sharing with the biomedical community. Together with Dr. Bishop they have extensive experience in developing data sharing approaches and providing informatics tools to the research community using visualization of data through web portals to facilitate viewing and data mining. Prior to publications, all omics data will be deposited in the Gene Expression Omnibus depository. Raw data and metadata will be exported and shared with other scientists. We will adhere to the NIH Grants Policy on Sharing of Unique Research Resources and make all analytical algorithms developed in this project available to the research community.

Mr. Miller and Drs. Bishop and Chen agree that bioinformatics tools, and any other metadata, collected will be widely shared with the scientific community for research and made publicly available through our data portal and other data repositories, such as Github and protocols.io. Software tools, if developed solely using this fund, will be freely distributed under GNU General Public License (GPL). Mr. Miller and Drs. Bishop and Chen also agree to cooperate with NIH staff, and other stakeholders in the development and implementation of research and standardization methods, data standards and formats, metadata requirements, and quality control metrics for this resource.

Any plasmids created and described in publication will be deposited in AddGene. Any other resources that we describe in publication will also be made available upon request to investigators at academic institutions for noncommercial research purposes. Requests for reagents from for-profit corporations will be negotiated by our institution’s Office of Technology Licensing. All licensing shall be subject to distribution, pursuant to my institution’s policies and procedures on royalty income. The Office of Technology Licensing will report any invention disclosure submitted to them to the appropriate federal agency.

Our institution and the various researchers involved in the proposal will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts (http://www.ott.nih.gov/policy/rt_guide_final.html). Specifically, material transfers would be made with no more restrictive terms than in the SLA or the UBMTA and without reach-through requirements. Should any intellectual property arise that requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

Beyond these requirements we will also deposit our data in other appropriate repositories within six months of the conclusion of the grant.

Sharing Model Organisms: No model organisms will be developed in the proposed research.

Genomic Data Sharing: All digital (for example, bioinformatics/sequencing) data will be made available for download at our Institute website (for example: Resources Section in Dr. Bishop (https://gccri.uthscsa.edu/lab/bishop/) or Dr. Chen Lab web page (https://gccri.uthscsa.edu/lab/chen/), or provided as a separate website as appropriate, similar to what we hosted at github (https://github.com/chenlabgccri, currently 6 projects were released along with shared source codes/data). Within the GCCRI we have adequate resources and support to implement the database storage and sharing.

In addition, through our GCCRI Bioinformatics group (UTH-SA), we will also store and provide our data through the databases and accessions such as Gene Expression Omnibus (GEO). Our bioinformatics group, with UTH-SA Department of Population Health Sciences, has implemented a clinical database system, Informatics Data Exchange and Acquisition System (IDEAS). Upon finishing the project or publication of research result, data and software will be released via GCCRI web site. ChIP-seq, RRBS, DRIP-seq, and any other datasets generated in this study will be deposited at GEO. Datasets will be from mouse cell lines and will not exceed 100 samples.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Cells lines are provided by David Sinclair. All cell lines used in this proposal will be subjected to short tandem repeat (STR) DNA profiling for cell line authentication. All cell lines will be maintained for no longer than 6 months before restarting with an earlier passage stock or verifying cell identity by STS testing or sequencing by Dr. Chen. We will use STR DNA profiling service with laboratory procedures for cell line authentication in order to comply with the guidelines set forth by the American Type Culture Collection (ATCC) SDO Workgroup ASN-0002 Standards document. http://www.celllineauthentication.com/references.html

All experiments will be conducted a minimum of three times. For validation of any effects in cell lines we will use at least four biological replicates. Additionally, cell line aging phenotypes will be verified by repeating the results previously published.

All plasmids that will be used, including RED-LasRR, in this proposal will be confirmed by direct sequencing of essential regions.

Only certified and company-validated antibodies will be purchased and used in the study.

For sgRNAs, 3 sequences will be selected and independently tested.