Sample Application for Research Training and Career Development Funding

Through the K99/R00 Pathway to Independence Awards, NIA supports exceptional postdoctoral researchers in completing the final years of their postdoctoral work and transitioning to a role as an independent scientist. Each award has two phases, the K99 phase supporting postdoctoral training, and the R00 phase supporting an independent research career.

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https://www.nia.nih.gov/research/training/k99-r00-sample-applications
Summary Statement

Program Contact: Max Guo

Application Number: 1 K99 AG065200-01A1

Principal Investigator
HIGUCHI-SANABRIA, RYO

Applicant Organization: UNIVERSITY OF CALIFORNIA BERKELEY

Review Group: NIA-B
Biological Aging Review Committee
NIA B

Meeting Date: 09/26/2019
Council: JAN 2020
Requested Start: 04/01/2020

Project Title: More than just a load control: cytoskeletal form and function during aging

SRG Action: Impact Score: 10

Next Steps: Visit https://grants.nih.gov/grants/next_steps.htm

Human Subjects: 10-No human subjects involved
Animal Subjects: 10-No live vertebrate animals involved for competing appl.

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<th>Project Year</th>
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<th>Estimated Total Cost</th>
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Administrative Budget Note: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the Committee Budget Recommendations section.
RESUME AND SUMMARY OF DISCUSSION: This resubmission application for a Pathway to Independence Award (K99/R00) from the University of California (UC), Berkeley, CA, on behalf of the candidate Dr. Ryo Higuchi-Sanabria, proposes to study novel genes that are found to alter the chromatin state and lipid homeostasis in regulating the actin cytoskeleton. The application will also examine the influence of neurons overexpressing the heat-shock factor 1 (hsf-1) on actin in peripheral tissues in addition to searching for differences in genes involved in actin changes among three peripheral tissues. Thus, this study may provide new and significant insights to our understanding of how and why the cytoskeleton changes occurs with advancing age. The area of study is significant, and the application is well written. The candidate has a strong trajectory and record of scientific accomplishments, from publishing to devotion to training and mentoring students. His mentoring team and the advisory committee are strong, helping the candidate navigate all aspects of the dependent and independent phases of the application. The research environment at UC Berkeley is superb, very supportive, and committed to the applicant’s career development. The resubmission was viewed as being very responsive and it was acknowledged that the candidate has done an outstanding job by responding to the concerns of the prior review by putting together a detailed career development and training plan. In response to the previous critique the candidate has now brought in Dr. Anne Brunet from Stanford, an excellent mentor in aging with a successful record of accomplishment of training fellows into independence. The research plan is now substantially shortened and streamlined. It has now two instead of three Aims, by essentially combining the previous Aims 1 and 2. There is now sufficient detail on the number of animals and statistical analysis, as well as a thorough discussion of caveats, pitfalls and alternative approaches. In summary, the review committee is enthusiastic about the candidate and the potential to develop as an independent scientist and make major contributions.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH: Acceptable. The training in the responsible conduct of research is adequately addressed.

DESCRIPTION (provided by applicant): Although the cytoskeleton has historically been understood as the structural framework of the cell, the proper function of actin is also required for a diverse array of cellular pathways. The collapse of these cellular processes manifests during aging and exposure to a myriad of stresses, which is in part due to the breakdown of the cytoskeleton under these conditions. Interestingly, the breakdown of the cytoskeleton throughout age has been adopted as common knowledge in the field of aging biology, despite the lack of clear and direct evidence. A major contributor to the lack of these essential studies is the lack of tools available for in vivo, live-cell imaging of the actin cytoskeleton in multi-cellular organisms. Early in my postdoctoral career, I developed a system for robust, tissue-specific, live-cell imaging of the cytoskeleton in the muscle, intestine, and hypodermis of C. elegans, utilizing LifeAct fused to a fluorescent molecule. LifeAct-mRuby reliably binds to F-actin, allowing visualization of functional, filamentous actin in the cells it is expressed. Using this system, I performed an exhaustive characterization of the decline of actin cytoskeletal integrity during aging. This work laid the foundation of my currently ongoing work in identification of novel regulators of the actin cytoskeleton. Having set up a system to interrogate cytoskeletal quality, I can now interrogate novel genes in their potential role for actin regulation. Using this and other platforms, I performed a multi-pronged screening approach to identify novel genetic regulators of actin. These studies combined in vivo live cell imaging of actin filaments, synthetic lethality screening with known regulators of the actin cytoskeleton, and both transcriptome analysis and whole genome CRISPR-Cas9 screening of organisms experiencing actin stress. Cross-referencing these rich datasets has revealed two critical nodes of genes: 1) modifiers of chromatin state and their downstream transcriptional regulators and 2) genes involved in lipid storage and global lipid homeostasis. In Aim 1.1, I hypothesize that a general chromatin state exists to promote a healthy transcriptome for proper cytoskeletal form and function, and that this breaks down as a function of age. Moreover, a healthy metabolic state can work either upstream of – or independent of – chromatin remodeling to also promote cytoskeletal health. In Aim 1.2, I propose to study whether any of the identified processes can function in a tissue-specific manner and a cell non-autonomous manner, by answering two questions:
1) is overexpression of chromatin remodeling or lipid homeostasis factors in a single tissue sufficient to preserve organismal lifespan? and 2) does overexpression of these genes in neurons drive protection of the actin cytoskeleton in peripheral tissue? Aim 2 uses 2 biochemical approaches to assess cytoskeletal function. First, proximity labeling will be used to characterize novel protein interactors of actin important for proper form and function. Second, we are building a tool for a biochemical approach for quantifying actin function with single cell resolution. This study will open exciting avenues of research in understanding the role of cytoskeletal function on physiological aging.

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

DISCLAIMER: Please note that the following critiques were prepared by the reviewers prior to the Study Section meeting and are provided in an essentially unedited form. While there is opportunity for the reviewers to update or revise their written evaluation, based upon the group’s discussion, there is no guarantee that individual critiques have been updated subsequent to the discussion at the meeting. Therefore, the critiques may not fully reflect the final opinions of the individual reviewers at the close of group discussion or the final majority opinion of the group. Thus, the Resume and Summary of Discussion is the final word on what the reviewers actually considered critical at the meeting.

CRITIQUE 1:

Candidate: 1
Career Development Plan/Career Goals/Plan to Provide Mentoring: 1
Research Plan: 2
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1
Environment, Commitment to the Candidate: 1

Overall Impact:
In this resubmitted K99 application, network of molecules involved in maintaining the cytoskeleton, and specifically actin, will be examined with the goal of understanding why the cytoskeleton deteriorates with advancing age in peripheral tissues. The application will also seek to identify molecular mechanisms responsible for the disparate rates of actin deterioration across different tissues during aging in c. elegans. Two aims are proposed and in Aim 1, a number of assays will be deployed to determine how chromatin remodeling and altered lipid homeostasis affect actin organization and function. This aim will primarily focus on BET-1, a bromodomain protein, role in modulating the actin cytoskeleton by stress and normal aging. In Aim 2, molecular tools will be optimized and developed to identify new modulators of the actin cytoskeleton and how it changes in specific cell types. Overall, the applicant did a great job of answering concerns previously raised making this line of inquiry all the more important and to be carried out by an applicant with immense promise as a researcher, teacher, mentor and long-term contributor to the biological sciences.

1. Candidate:
Strengths
• BA from Hunter College, MA and PhD with 2015 graduation from Columbia in Nutrition and Metabolic Sciences, and postdoctoral fellowship at UC Berkeley all on the biological sciences.
• The candidate has demonstrated a clear commitment to research, to mentoring the next generation of scientists, particularly from underrepresented groups, at all career stages.
• Has published 10 manuscripts to date and all as first or co-first author.

**Weaknesses**
• None noted.

2. Career Development Plan/Career Goals & Objectives:

**Strengths**
• Instructional courses on Mass Spectrometry at the Proteomic core at UC Davis and Databases and Tools of Bioinformatics at UC Berkeley.
• Collaboration with aging researchers and frequent attendance at aging-related meetings.
• Strong team of mentors, starting with primary mentor Dr. Andrew Dillin having the expertise, track record and clear devotion to help advance the candidate’s career. The mentoring team - consisting of Drs. Eisen who is an expert geneticist and on computational approaches, Drubin expert on the cytoskeleton, Ting who has developed novel methods for protein labeling and purification, Brunet expect aging biologist and with extensive knowledge of chromatin structure, and Herr who brings expertise in single-cell immunoblot.

**Weaknesses**
• None noted.

3. Research Plan:

**Strengths**
• There is strong rational for studying BET-1 function and potential dependence on histones acetylated by MYST HATs is strong. Importantly, the appropriate experiments are proposed to establish relationships between the various molecules and changes in the actin cytoskeleton.
• The experiments to determine the impact of aging on BET-1 levels and function are critical for determining the contribution of this transcription factor to age-related changes along the cytoskeleton.
• Using a variety of cellular, molecular and biochemical tools to uncover new interactors and modulators of actin, including those affected by changes due to lipid composition.
• The development of new tools to study the cytoskeleton with more precision in specific cells and tissues.

**Weaknesses**
• The use of single cell western blotting does not appear to generate additional and valuable information regarding mechanisms involved in altering actin, beyond establishing that the F:G actin ratio declines with increasing age in various cells in c. elegans.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

**Strengths**
• Dr. Andrew Dillin is the primary mentor. He is a world-renowned expert on aberrant molecular changes that contribute to the normal aging process, particularly assessing basic yet critical mechanisms. He is very supportive of the applicant, application and path to independency. The co-mentors and collaborators bring expertise in all other areas necessary for this candidate and are very supportive of the applicant and application. Dr. Michael Eisen, an HHMI investigator, is renowned for using biochemical approached to study the transcriptional state of chromatin; Dr. David Drubin is expert on the cytoskeleton; Dr. Alice Ting has developed new tools for imaging and biochemical studies and will help with miniTurbo; Dr. Anne Brunet is an expert aging biologist; and Dr. Amy Herr brings bioengineering expertise for the last subaims of this application. Altogether, this team of mentors and collaborators have vast experiences and expertise and importantly the desire to support this applicant during the K and R portions of this application.

**Weaknesses**
• None noted.
5. Environment and Institutional Commitment to the Candidate:

**Strengths**
- The MCB department at the University of California, Berkeley is excellent environment.

**Weaknesses**
- None noted.

Training in the Responsible Conduct of Research:

Acceptable.

**Comments on Format:**
- Appropriately described for both formal and informal training.

**Comments on Subject Matter:**
- Appropriate.

**Comments on Faculty Participation:**
- A plan is in place for the primary mentor and co-mentors to advise on best practices on research and teaching.

**Comments on Duration:**
- Appropriate.

**Comments on Frequency:**
- Weekly meetings.

Resource Sharing Plans:

Acceptable.

Authentication of Key Biological and/or Chemical Resources:

Acceptable.

Budget and Period of Support:

Recommend as Requested.

CRITIQUE 2:

Candidate: 1

Career Development Plan/Career Goals/Plan to Provide Mentoring: 2

Research Plan: 1

Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1

Environment, Commitment to the Candidate: 1

Overall Impact:

This K99-R00 resubmission is from UC Berkeley, by a talented postdoctoral fellow, Dr. Higuchi-Sanabria, who studies cytoskeletal actin biology during cell stress and aging. The candidate proposes to advance his research and training in actin cytoskeleton regulation by identifying and interrogating genes that impact chromatin state and lipid homeostasis. Previous concerns of the career development training have been addressed by including plans for attending workshops and meetings on aging as well as engaging in Glenn Foundation activities and adding plans for scientific skill development. Concerns in the research plan are addressed by better detailing study designs and including alternative hypotheses/experiments/methods and focusing the aims, largely by removing the previous aim on hsf-1. The mentors and environment remain superb and fully support the development of this emerging, gifted scientist toward an independent research career to study how cytoskeletal maintenance deteriorates during aging.

1. Candidate:
Strengths

- The candidate has a strong background in cell biology research from his doctoral work at Columbia University where he studied actin cytoskeletal dynamics and mitochondrial contributions to lifespan. Thus, his research is rooted in aging biology.
- Productivity is solid with 9 publications on PubMed, with seven first author publications from his doctoral research, all in prestigious journals.
- Variety of experiences in teaching and mentoring demonstrate conviction to science education and academia along with research.
- The candidate has been successful in advancing imaging technologies for yeast, mitochondrion, and actin as evidence by methodological publications and publications in which the techniques have been utilized.
- Mentoring experience (2 graduate and 3 undergraduate students, rotating students, etc.) is providing a strong platform for career independence in the R00 phase as do his previous and current teaching experiences.

Weaknesses

- None noted.

2. Career Development Plan/Career Goals & Objectives:

Strengths

- The candidate proposes to acquire new skills and knowledge, especially in analyses of large sequencing, bioinformatics, and proteomic datasets. This will be done by through mentorship of experts Drs. Meyer, Ting, Phinney, and Eisen.
- Strengthening his biochemistry acumen will be accomplished with mentorship from Drs. Drubin and Herr.
- The candidate will take good advantage of opportunities available through the Glenn Foundation to enhance his aging research agenda.
- Long term goals now clearly delineate an interest and intent in aging of cytoskeleton stress response and how that intersects with other organelles and their functions.

Weaknesses

- An actin biochemist on the advisory team or some type of direct mentoring in actin biochemistry would add value.

3. Research Plan:

Strengths

- Application of new live cell imaging in concert with transcriptome analysis and whole genome screening is a powerful approach in aim 1 to further interrogate actin regulation by chromatin remodeling and lipid homeostasis.
- Cytoskeletal biochemistry evaluation across tissues and at the single cell level and how those change with age in aim 2 will contribute new knowledge to the biology of aging field.
- Identification of genes that modify chromatin state and those that are involved in lipid storage and homeostasis by overlapping results from two independent screens lays solid foundation for the work proposed.
- Alternative approaches (caveats) are described for each subaim should results not support the original hypotheses.
- Relegating the lipid homeostasis studies to the R00 phase is a wise choice and should help establish sovereignty for the candidate in regard to launching his independent laboratory.

Weaknesses

- Minor concern that research proposed is similar enough to mentors “The Collapse of Proteostasis during Aging is Mediated by Cytoskeletal Actin Functions” such that separation at the R00 phase should be further detailed.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):
Strengths
- Dr. Dillin is the primary mentor; he is an HHMI investigating aging and age-related diseases. He has exceptional mentoring experience with several trainees progressing to independence in their science careers.
- The mentor has solid funding spanning past the K99 phase of the application.
- The advisory committee and new co-sponsor Dr. Meyer add strengths in biochemistry training and –seq type analyses as well as advisors in aging biology (Drs. Brunet and Zoncu).

Weaknesses
- None noted.

5. Environment and Institutional Commitment to the Candidate:
Strengths
- Detailed plans are in place to assist the candidate in the faculty search process ranging from the mentor assisting with application/interview/negotiation processes to equipment procurement.
- The overall Drs. Dillin and Meyer and the environment at Berkeley is superb.

Weaknesses
- There is no mention of the Berkeley’s Postdoctoral Association which may broaden the candidate’s perspective on career development in academia and non-academic avenues; very minor concern.

Resubmission:
- The candidate has been very responsive to the previous critiques particularly the major concerns in the training and research plans.

Training in the Responsible Conduct of Research:
Acceptable.

Comments on Format:
- Formal and informal face-to-face and CITI online courses.

Comments on Subject Matter:
- Conflict of interest; ethical use of animals; history of humans and research; data management and access to research tools; responsible conduct of research; mentorship; authorship and publication; stem cell research.

Comments on Faculty Participation:
- Dillin (sponsor) will participate.

Comments on Duration:
- Formal 5 x 2 hr. meetings.

Comments on Frequency:
- Approximately every 3 years.

Resource Sharing Plans:
Acceptable.

Authentication of Key Biological and/or Chemical Resources:
Acceptable.

Budget and Period of Support:
Recommend as Requested.

CRITIQUE 3:
Candidate: 1
Career Development Plan/Career Goals/Plan to Provide Mentoring: 2  
Research Plan: 1  
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1  
Environment, Commitment to the Candidate: 1

Overall Impact:
This is a resubmission of a K99 application by a talented postdoc, Dr. Ryo Higuchi-Sanabria, to study actin and the cytoskeleton in the nematode worm during aging. Strengths of the application are the scientific excellence of both the applicant and the proposed mentor, Dr. Andrew Dillin, and the exciting and very well written research plan. Weaknesses noted the first time around, e.g., the thin career development plan with few details on evaluation, metrics and course work and no formal training in the biology of aging, and the overambitious research plan with a lack of detail on statistics, caveats and alternative approaches, has been effectively remedied.

1. Candidate:
Strengths
- Dr. Ryo Higuchi-Sanabria received his PhD from Columbia University in 2015 on yeast mitochondrial dynamics in the lab of Dr. Liza Pon. Since 2016 he is a postdoctoral fellow in the lab of Dr. Andrew Dillin, University of California, Berkeley, the primary mentor of the proposed study.
- Dr. Higuchi has been productive with 6 first-author papers since 2013, one with his current mentor, Dr. Dillin, in 2018, a review in Developmental Cell, also in 2018 with Dr. Dillin.
- Dr. Higuchi is the PI on an F32.
- Reference letters are very good.

Weaknesses
- None noted.

2. Career Development Plan/Career Goals & Objectives:
Strengths
- Dr. Higuchi is committed to a career in studying cytoskeletal homeostasis in relation to aging. Both his background at Columbia and his research plans are very well described. The same is true for his planned interactions with committee members, Drs. Eisen, Ting and Drubin, and his plans for training students.
- A previously noted weakness, a thin career development plan with no formal training in the biology of aging, has now been remedied.

Weaknesses
- None noted.

3. Research Plan:
Strengths
- Actin as a critical part of the cytoskeleton and associated proteins is generally assumed to breakdown with age, but this is understudied, in part due to a lack of tools for in vivo, live-cell imaging of the actin cytoskeleton in multi-cellular organisms. The candidate developed a system for tissue-specific, live-cell imaging of the cytoskeleton in C. elegans, based on a peptide, termed LifeAct, that binds specifically to F-actin. This has been fused to mRuby, a fluorophore, allowing visualization of functional, filamentous actin. This and other tools allow the proposed studies of HSF-1 dependent and independent regulation of actin organization and the identification of novel regulators of the actin cytoskeleton. The proposed study is significant and, based on the tools developed, likely to increase our understanding of actin and cytoskeleton breakdown during aging of the worm.
• Based on the candidates from the screen, in Aim 1 the applicant proposes to characterize the molecular pathways associated with these genes, i.e., chromatin modifiers and lipid metabolism, and how they contribute to cytoskeletal integrity.

• Aim 2 makes use of available expertise in proximity-based protein labeling in the lab of Dr. Ting, a member of the advisory committee. The proposed combined application of the improved miniTurbo system and the LifeAct probe to characterize protein interactors of actin important for its proper form and function is innovative and could yield important novel insight.

Weaknesses
• None noted.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):
Strengths
• Dr. Andrew Dillin, the proposed mentor is a Professor in the Department of Molecular and Cell Biology, University of California at Berkeley, since 2012. His research focus is on ER proteostasis and aging. Dr. Dillin is renowned in the field of aging, extremely well published and very well-funded through the NIH. He is the best possible mentor for the proposed project.
• The advisers, Drs. Eisen, Drubin, Phinney and Ting are all very good and provide unique and critical technical support.

Weaknesses
• None noted.

5. Environment and Institutional Commitment to the Candidate:
Strengths
• The Dillin lab in the Li Ka Shing Center on the University of California, Berkeley campus is a great environment for the proposed project.
• Institutional support is testified by the letter from Dr. Rio, co-chair of the Department of Molecular and Cell Biology.
• There is now a formal connection in the application to the Glenn Centers for Biology of Aging in the Bay Area.

Weaknesses
• None noticed.

Training in the Responsible Conduct of Research:
Acceptable.

Resource Sharing Plans:
Acceptable.

Authentication of Key Biological and/or Chemical Resources:
Acceptable.

Budget and Period of Support:
Recommend as Requested.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS’ WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 K99 AG065200-01A1; PI Name: Higuchi-Sanabria, Ryo
NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.