Presentation of
Rigor and Reproducibility in NIH Grants

Scientific Writing in Rehabilitation Science
Department of Physical Therapy & Rehabilitation Science
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• Laboratory researcher for 14 years
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• Expanded to CCOM 2017
Activities:

- Provide input on drafts of writing projects
- Provide consultation on writing strategy
- Teach scientific writing
- Brainstorm with authors on projects
- Collect and generate resources
  - Changes in funding agency requirements
  - Grant writing templates (NIH “R” and “F” grants)
- Liaise with other RD professionals

Strategy for writing the Specific Aims page...

Opening sentence
Current knowledge
Gap in knowledge/why it matters
Long-term goal
Objective (proposed research)
Central hypothesis (or urgent need)
Rationale for study
Aims Title
Hypothesis of Aim
Expected outcomes
Broader impact
Impact on career goals

https://medicine.uiowa.edu/sercc/resources/writing-grants
Rigor and Reproducibility in NIH Grants

NIH Instructions
Fellowship Research Strategy
Rigor and Reproducibility

How to Structure the
Research Strategy

Examples of Wording
Rigor & Reproducibility
Other Aspects

Questions?

Example of funding agency expectations
NIH Individual Predoctoral Kirchstein NRSA Fellowships

Research Training Plan:
1) Specific Aims page, no > 1 page (include 2–4 aims)
2) Research Strategy, no > 6 pages
   a. Significance
   b. Approach
Changes in NIH Requirements Since 2010

- **2010** – for all grant mechanisms
  - Shortened Research Strategy by 50%
  - “Background and Significance” → “Significance”
  - “Innovation” section added (for “K” and “R” mechanisms)

- **2016** – for K and R mechanisms
  - Required evidence of rigor and reproducibility, including:
    - Discussion of *scientific premise* within Significance section
    - Discussion of *rigor of proposed research* in Approach
    - Discussion of *biological variables* in Approach
    - Explanation of *how key resources will be authenticated* (attachment)

- **2019** – for K and R mechanisms
  - Changed *scientific premise* to *weaknesses in rigor of prior research*
  - Requires discussion of *how weaknesses in rigor of prior research will be addressed* in Approach

### NIH definition of Scientific Rigor (2018)…

- **the strict application of the scientific method**
- **to ensure unbiased and well-controlled**
  - experimental design
  - methodology
  - analysis
  - interpretation and
  - reporting
- **of results**

Current NIH Instructions

A careful assessment of the rigor of the prior research that serves as the key support for a proposed project will help applicants identify any weaknesses or gaps in the line of research.

- In Significance section:
  Describe the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.

- In Approach section:
  Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project.
Adapting to change

- SERCC: Are new requirements addressed in the right places?
  - **Significance**
    - Weaknesses in rigor of prior research
  - **Approach**
    - How weaknesses in rigor of prior research will be addressed
    - How rigor of proposed research will be ensured
    - Consideration of biological variables, including sex, in the proposed research
- Division of Sponsored Programs:
  - Are requirements addressed?
    - (Will return application if Authentication page is missing)

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Example of funding agency expectations

**NIH Individual Predoctoral Kirchstein NRSA Fellowships**

**Research Training Plan:**

1) Specific Aims page, no > 1 page (include 2–4 aims)
2) Research Strategy, no > 6 pages
   a) **Significance**: Describe...
      - the importance of the problem or critical barrier to progress that the proposed project addresses.
      - the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.
      - how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
      - how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.
   b) **Approach**

Green text: relevant to Rigor & Reproducibility
Example of funding agency expectations
NIH Individual Predoctoral Kirchstein NRSA Fellowships

a) Significance

b) Approach: Describe…

• The overall strategy, methodology, and analyses to be used…

• Potential problems, alternative strategies, and benchmarks for success

• If the project is in the early stages of development, any strategy to establish feasibility/address management of any high risk aspects

• How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans

• Any procedures/situations/materials that may be hazardous to personnel and the precautions to be exercised

• If research on Human Embryonic Stem Cells (hESCs) is proposed but an approved cell line from the NIH hESC Registry cannot be chosen, strong justification for why.

• If you are proposing to gain clinical trial research experience (i.e., you will not be leading an independent clinical trial), your role on the clinical trial

Blue text: relevant to Rigor & Reproducibility
Topics

NIH Instructions
Fellowship Research Strategy
Rigor and Reproducibility

How to Structure the Research Strategy

Examples of Wording
Rigor & Reproducibility
Other Aspects
Questions?

Our grant writing templates…

https://medicine.uiowa.edu/sercc/resources/writing-grants
Research Training Plan:
1) Specific Aims page, no > 1 page (include 2–4 aims)
2) Research Strategy, no > 6 pages
   a) Significance: Describe…
      • the importance of the problem or critical barrier to progress that the proposed project addresses.
      • the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.
      • how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
      • how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.
   b) Approach

Example of funding agency expectations
NIH Individual Predoctoral Kirchstein NRSA Fellowships

Significance – Our Recommendations 2019

Importance of the problem and/or critical barriers to progress
Scientific premise and rigor of the prior research *
Significance of the expected research contribution
• Impact of the project on scientific knowledge / technical capability / clinical practice
• Impact of the project on the field

* The relevant literature: Strengths and weaknesses
• Rigor of study design (e.g. statistical power, blinded analysis)
• Incorporation of relevant biological variables (e.g. detail regarding sex)
Your preliminary data that contribute to scientific foundation of proposal.
Significance Section...

- Importance of Problem
- Scientific Premise and Rigor of Prior Research
- Significance of Expected Research Contribution
  - impact on scientific knowledge
  - impact on the field

Example of funding agency expectations

**NIH Individual Predoctoral Kirchstein NRSA Fellowships**

a) Significance

b) Approach: Describe...

- The overall strategy, methodology, and analyses to be used, including...
- Potential problems, alternative strategies, and benchmarks for success
- If the project is in the early stages of development, any strategy to establish feasibility/address management of any high risk aspects
- How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans
- Any procedures/situations/materials that may be hazardous to personnel and the precautions to be exercised
- If research on Human Embryonic Stem Cells (hESCs) is proposed but an approved cell line from the NIH hESC Registry cannot be chosen, strong justification for why
- If you are proposing to gain clinical trial research experience (i.e., you will not be leading an independent clinical trial), your role on the clinical trial

Blue text: relevant to Rigor & Reproducibility
Structure for *Approach* section – previously

**Approach**
- For each aim
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph (including background and preliminary data)
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies paragraph
- Timeline and Benchmarks for success
- Future Directions

Structure for *Approach* section – new

**Approach**
- **Issues related to rigor and reproducibility**
  - Addressing weaknesses in rigor of prior research
  - Strategies to ensure rigor of proposed research
  - Consideration of biological variables including sex
- **Aim x (for each aim)**
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph (including background and preliminary data)
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies paragraph
- Timeline and Benchmarks for success
- Future Directions
Approach – Our Recommendations 2019

Approach

➢ Issues related to rigor and reproducibility
  • Addressing weaknesses in rigor of prior research
  • Strategies to ensure rigor of proposed research
  • Consideration of biological variables including sex

➢ Aim x (for each aim)
  • Title of Specific Aim
  • Introduction/rationale paragraph
  • Justification and Feasibility paragraph
    (including background and preliminary data)
  • Research Design paragraphs
  • Expected Outcomes paragraph
  • Potential Problems and Alternative Strategies paragraph

➢ Timeline and Benchmarks for success

➢ Future Directions

Structure for Approach section – new

Approach

➢ Issues related to rigor and reproducibility
  • Addressing weaknesses in rigor of prior research
  • Strategies to ensure rigor of proposed research
  • Consideration of biological variables including sex

➢ Aim x (for each aim)
  • Title of Specific Aim
  • Introduction/rationale paragraph
  • Justification and Feasibility paragraph
    (including background and preliminary data)
  • Research Design paragraphs
  • Expected Outcomes paragraph
  • Potential Problems and Alternative Strategies paragraph

➢ Timeline and Benchmarks for success

➢ Future Directions

1. Rigor of proposed research → robust, unbiased results (discuss any of the categories below that apply)
  • Randomization protocol for sample groups, inclusion/exclusion criteria
  • Blinded data recording and analysis
  • Controls and replicates needed
  • Sample-size estimation/power analysis (critical for studies using human subjects and higher vertebrates)
  • Principles of good laboratory practice
  • Essential reagents and their authentication
  • Statistical analyses to be used
  • Controls and replicates needed

2. Relevant biological variables including sex
  • Sex (equal numbers of each; impact on results; separate analysis of effects; karyotype of cell lines)
  • Weight, age, health status, body mass index, underlying comorbid conditions...
Approach Section...

Topics

NIH instructions
Fellowship Research Strategy
Rigor and Reproducibility

How to Structure the Research Strategy

Examples of Wording
Rigor & Reproducibility
Other Aspects
Questions?
Significance – Our Recommendations 2019

1) Importance of the problem and/or critical barriers to progress

2) Scientific premise and rigor of the prior research (can organize overall or by aim)
   • Numerous studies have…
   • However, studies X and Y have important limitations…
   • In addition, the rigor of study Z was not sufficient in that…
   • To overcome these gaps in rigor, we will… [keep this general here]
   • Thus, our proposed studies will circumvent the limitations of… by …

3) Significance of the expected research contribution
   • Impact of the project on scientific knowledge / technical capability / clinical practice
   • Impact of the project on the field

Example of Strategies to Ensure Rigor (from our authors)

R37 Renewal, scored in 2nd percentile – New subsection (after Aim 3)

Research Rigor and Transparency: Scientific rigor and reproducibility is maintained when opportunities for error are minimized through education of the team members about potential sources of error. To this end, the PI, staff, and students consult a Biostatistics and Research Design Core within the UI Institute for Clinical and Translational Sciences in the methodological planning of research protocols. This ensures robust statistical outcomes and post-experimental analysis of data. The PI and all associated personnel have also received NIH-mandated ethics training. All data will be reviewed by multiple team members to ensure its validity and to minimize operator biases; this occurs formally at twice weekly lab meetings, informally between trainees and the PI, and at the time of manuscript preparation, when the PI reviews all the raw data files. Morphometric analysis will be performed by blinded teams of students. Inbred C57BL/6 strains will be used, with the exception of CF mice for which sibling CF and WT or heterozygous animals will be compared as previously described.

Key points:
• Multiple approaches used to test each hypothesis.
• Multiple steps in process of data review and analysis ensure validity and minimize author bias.
• The rigor of the scientific approach is outstanding.
Examples of Strategies to Ensure Rigor (posted by NIH)

- Excerpts from awarded applications reviewed under a pilot FOA for rigorous experimental design ... this is only one part of updated instruction and review language.
- Selected based on high overall impact scores and positive reviewer comments specific to rigor.
- Provided to show how elements of rigor and transparency have been succinctly provided in applications; they may not represent all of the aspects/may still have room for improvement.
- May be updated as applications are reviewed and awarded under the revised rigor and transparency review.

Example 1:

**Aim 3**: Male and female mice will be randomly allocated to experimental groups at age 3 months. At this age the accumulation of CUG repeat RNA, sequestration of MBNL1, splicing defects, and myotonia are fully developed. The compound will be administered at 3 doses (25%, 50%, and 100% of the MTD) for 4 weeks, compared to vehicle-treated controls. IP administration will be used unless biodistribution studies indicate a clear preference for the IV route. A group size of n = 10 (5 males, 5 females) will provide 90% power to detect a 22% reduction of the CUG repeat RNA in quadriceps muscle by qRT-PCR (ANOVA, α set at 0.05). The treatment assignment will be blinded to investigators who participate in drug administration and endpoint analyses. This laboratory has previous experience with randomized allocation and blinded analysis using this mouse model [refs]. Their results showed good reproducibility when replicated by investigators in the pharmaceutical industry [ref].

**Key points:**
- Number of groups, allocation random, age, why that age.
- Dosage, number of doses administered
- Route of administration, contingency
- Group size, power
- Blinding, of whom
- Experience

Examples of Strategies to Ensure Rigor (posted by NIH)

Example 2:

**Aim 1**: Primary screen: In this high throughput screening assay, we combined the SMN promoter with exons 1-6 and an exon 7 splicing cassette in a single construct that should respond to compounds that increase SMN transcription, exon 7 inclusion, or potentially stabilize the SMN RNA or protein [refs]. The details of the assay and the SMN2-luciferase reporter HEK393 cell line have been extensively validated [refs]. Each point is run in triplicate, the compounds are tested on three separate occasions, and the results are averaged to give an EC50 with standard deviation. Secondary screen: ... We analyze SMN protein levels by dose response in quantitative immunoblot analysis with statistical analysis by one-way ANOVA with post-hoc analysis using Dunnett or Bonferroni, as appropriate.

**Aim 2**: Each set of compounds will include a blinded negative control compound that has been determined to be inactive and that is solubilized in the same manner as test compounds. Mice will be randomly assigned within a litter, and data will be collected and submitted to the PI. For compounds that demonstrate extended survival, the PI will be sure to have these tested in (the collaborators') lab, and data will be merged and evaluated. To calculate the number of the experimental mice, we will perform an SSD sample size power analysis to ensure that the appropriately minimal number of mice is used in each experimental context. Typically for each compound in life span studies, we will need ~20 SMA animals in the treated group; ~20 SMA animals in the vehicle treated group; ~20 SMA animals in the untreated group. If we can administer the compound in aqueous solution without expedient, the vehicle and untreated groups might be combined, as these should have identical survival. Therefore, no more than 80 SMA animals will be needed per compound.

**Key points:**
- Brief summary of overall approach
- Number of replicates, same/different dates, reporting of average with standard deviation
- Types of statistical analysis

**Aim 2**: Blinding, solubilization of test and control compounds
- Random assignments
- Who will analyze
- Power analysis; number of animals per group
- Number of animals, contingency
Example 3:
Aim 2: Intensity signal data will be transformed into log values and then modeled by longitudinal methods (reference cited). Specifically, the composite difference in mean intensity signals over time between the bi-specific T cells vs. control groups is assumed to be 2.8 logs with a composite standard deviation of 2.2 logs. Furthermore, we will assume at least five repeated measurements per mouse after T cell infusion and a within-mouse intra-correlation coefficient equal to 0.50. Thus, a sample size of 10 mice per group will provide at least 80% power to detect the above difference between treated versus control group with a 5% significance level. Log-rank test will be used to compare the survival distribution between groups. VAS: Animal numbers are based on the requirement to perform each experiment (power and sample size calculations are described in the Research Strategy), which includes an independent experimental repeat.

Example 4:
Aim 1: Statistical considerations: In our preliminary studies consisting of this same cohort of DFUs (n=100) and utilizing 16S rRNA sequencing, we were able to detect dimensions of DFU microbiome, including microbial diversity, that were significantly associated with DFU outcomes. We therefore anticipate that the sample size will provide sufficient power to detect significant differences using metagenomic sequencing, as this is a more sensitive and less-biased assay of microbial identification and diversity.

Key points, Example 3:
• Methods for conversion of signal data and modeling
• Number of measurements and assumptions made for power analysis
• Statistical measures to be used
• Numbers of animals needed; to be determined independently for each experiment

Key points, Example 4:
• Statistical considerations based on preliminary data
• Anticipated power of sample size for new, more sensitive assay
• Statistical measures to be used

Rigor and Reproducibility
https://grants.nih.gov/reproducibility/index.htm

Consideration of Sex as a Biological Variable (SABV)

Reviewer Guidance to Evaluate Sex as a Biological Variable (SABV)

Main points
• NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies.
• Animal and human studies may be analyzed as a whole, or separately, if justified by scientific rationale.
• In studies designed to detect sex differences, male and female groups must be compared in a blinded fashion.

1. Does the study imply vertebrate animals or human?

No further consideration of SABV required; not considered a weakness.

Yes

2. Is the study intended to test for sex differences?

No

Yes

3. Are both sexes included in the study?

No

Yes

4. Describe the proportional distribution of males and females.

No

Yes

Adequate consideration of both sexes in experiments and disaggregation of data by sex allows for sex-based comparisons and may inform clinical interventions.

Appropriate analysis and transparent reporting of data by sex may therefore enhance the rigor and applicability of preclinical biomedical research.

NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex. Investigators are strongly encouraged to discuss these issues with NIH program staff prior to submission of applications.

Further information regarding NIH expectations for the consideration of sex as a biological variable is provided at the following website:

Rigor and Reproducibility
https://grants.nih.gov/reproducibility/index.htm
Methods to achieve robust and unbiased results:
… and WT littermate controls were generated as described in Fig. 1. These lines were genotyped and cataloged across 10 backcrosses into the C57BL/6J strain. Only animals that are of the same genetic background and handled in the same way will be compared. Congenic Xxxx KO mice (B6.129P2-xxxxx/J; stock #xxxx) were obtained from Jackson Laboratories. These mice had been backcrossed with C57BL/6J animals >30 generations. For cultures of dissociated PFC cells obtained from neonates, there is no reason to think that gender differences exist; hence male and female pups will be randomly allocated to experimental groups at P1. For the experiments involving [brain] slices from P30 animals, samples will be prepared from equal numbers of age-matched male and female animals and results will be tracked by gender. Each experiment will be performed in triplicate and repeated at least three times. Dose-response and time-course analyses will be conducted for each compound to ensure that the responses are maximal. We have extensive experience with blinded analysis, treatment paradigms, and group analyses. The Co-Investigator has extensive experience in establishing LTP and LTP-D paradigms in both rats and mice. Experimental designs are rigorously vetted including, at a minimum, testing of only a priori hypotheses and blinding for subjective ratings. Except as noted, biological and chemical resources will be obtained from standard commercial suppliers; effects of novel agents are documented in the literature. Data will be analyzed using ANOVA followed by posthoc testing with Student’s t-test.
Approach

Issues related to rigor and reproducibility
- Addressing weaknesses in rigor of prior research
- Strategies to ensure rigor of proposed research
- Consideration of biological variables including sex

Aim x (for each aim)
- Title of Specific Aim
- Introduction/rationale paragraph
- Justification and Feasibility paragraph
  (including background and preliminary data)
- Research Design paragraphs
- Expected Outcomes paragraph
- Potential Problems and Alternative Strategies paragraph

Timeline and Benchmarks for success
Future Directions

Suggested structure for Approach section

Introduction paragraph, formula:

- Justify why this aim needs to be performed / what aspect of the overall problem will be addressed (1–2 sentences only)
- Explicitly state the objective, e.g. “The objective of this aim is to…”
- Restate (verbatim) the working hypothesis from Specific Aims page, e.g.: “To attain the objective of this aim we will test the working hypothesis that…”
- State the overall strategy / approach for testing hypothesis (1–2 sentences)
- Provide rationale for work under this aim (i.e. what will become possible after this work is carried out)
- Summarize overall outcome and positive impact of this aim, at a general level
Suggested structure for Approach section

Justification (& Feasibility) paragraph:

• Will potentially include two kinds of information
  • Justification of need — based on the literature
  • Evidence that you can do the work necessary to solve the problems you have framed — preliminary data

• If so, you need a good transition that bridges justification of need to evidence of feasibility, e.g.:

  These findings illustrate that … identifying x will be necessary to understand … , which will require knowledge of …. The following preliminary data support the feasibility of this approach in our hands.

Suggested structure for Approach section

Research design paragraphs:

• Write paragraphs related to research activities that will be undertaken to accomplish the objectives of that aim.

• In each paragraph, make a single conceptual point.

• Start each research activity off with an interest-grabbing headline.

  Aim 2.1. Determine which cells require the Mmd protein
  Approach / methods overview / essential reagents / critical equipment / numbers of subjects/animals and how numbers were derived
  statistical analysis to be used / controls / replicates / detailed expectations / how results will be interpreted / any major anticipated problems / time to complete

  Aim 2.2. Identify the mechanism whereby TXS activity repositions Mmd
  Approach / methods overview / essential reagents / critical equipment …
In writing research activities for each aim:

- Emphasize concepts
- Avoid anything tangential

For methodologies:
[as a student, you may need to include more detail than more senior researchers]

- reference any appropriate papers by your research team;
- if nobody on team has published with a certain methodology, then include preliminary data on this;
- refer to earlier descriptions of protocols where they are repeated.

**Suggested structure for Approach section**

Expected Outcomes paragraph (very important!):

- Purpose – Highlight expected return on the agency’s investment more explicitly than in summary or in introductory paragraph for the aim.
- In this paragraph you should:
  - summarize expected outcomes for this aim (one per activity)
  - convey how outcomes collectively achieve the objective of the aim
  - underscore importance of this activity to:
    - the field, of its own accord
    - the overall objective of this aim
  - mention any important caveats
Expected Outcomes paragraph (very important!):

- Example language for this —

These experiments will provide the first analyses of … Biochemical analyses will provide …, yielding a level of knowledge that has not been achieved in other systems. Combining this information with pharmacologic perturbations … will yield insight into the function of … will also provide insight into … Thus, the results will provide a foundation for attaining the overall objective of the proposal, i.e. …

Potential Problems and Alternative Strategies paragraph:

- Identify problems that could arise but probably won’t; only the most important and probable, e.g.:
  - if assays are not sufficiently discriminating
  - if critical reagents/patient samples are not available
  - if your working hypothesis is proven invalid

- For each, identify:
  - nature of the problem
  - reasons it is unlikely to arise
  - alternative approaches you would try if it were to arise
Potential Problems and Alternative Strategies paragraph:

- Example of language for this:

  Regardless of our hypothesis, the experiments within this aim will \ldots, and whether \ldots Given that the experiments within this aim use well established and routine genetic tools to determine \ldots, it is unlikely that the experimental techniques will fail. However, if \ldots the interpretation of the results could be problematic. To overcome such a complication, we would \ldots by \ldots For instance, our preliminary data indicates that\ldots Thus we would use \ldots to identify which \ldots.

Suggested structure for Approach section

Timeline

- Comes after all of the Specific Aims
- Purpose — outline timeframe needed to complete each subaim (table or paragraph)
  - Grant applications are often rejected because overly ambitious.
  - Grant applications are often rejected because not ambitious enough.
  - Carefully thinking through and presenting a timeline (preferably including a schematic component) can circumvent these problems.
Suggested structure for Approach section

**Future Directions**

**Purpose** — to summarize:

- where you expect the science to be at end of the study
- how the results will complete next step in the continuum projected in *long-term goals* statement of Specific Aims page
- what the next steps in the continuum will be, and why they are important

Example of language for this:

*... The knowledge gained will provide the foundation for ... In the future we plan to probe the mechanisms of ... by ... We will also expand the studies outlined here to examine whether ... Overall, such studies will help us attain our long-term goal of ... by ...*
Suggested structure for Approach section

EXAMPLE PROPOSAL 3
Risk Factors for Glaucoma: Uncovering their Genetic Basis Using Inbred Mouse Strains

complication, the selection of mice used in the porcyping arrays would become more tightly limited to mice representing only the extreme phenotypes — significantly high and low CEC density—which would increase the likelihood of identifying relevant loci. Another way to overcome this problem, would be the replacement of one strain with a different mouse strain of the CEC phenotype. This would be possible because we initially purchased many different strains of CEC density.

Timeline

Risks/Tasks | Year 01 | Year 02
---|---|---
1.4 Identify risk factors influencing CEC density | | |
1.5 Generate cohorts of intercrossed F2 progeny | | |
1.6 Phenotypic selection for CEC density and CEC | | |
1.7 Map genes to influencing CEC density | | |

Future Directions
The identification of genetic loci that influence CEC density greatly contribute to our understanding of the pathogenesis of glaucoma. An understanding of the genetic basis of CEC density is crucial to the current state of measuring CEC density in the clinical setting. Our ability to assess risk for disease, treat disease, and prevent vision loss associated with glaucoma would be greatly enhanced. Furthermore, genetic manipulation of low risk influence CECs as well as pharmacological studies aimed at manipulating CEC density would also be pursued for new preventive measures and treatments for glaucoma and other CEC-related diseases. This early detection would enable us to identify high-risk individuals, begin preventive treatments well before the onset of disease begins, and ultimately give us the opportunity to significantly reduce the incidence of vision loss associated with glaucoma.

Suggested structure for Approach section

approaches by selecting a subset of mice at postoperative days 7, 14, and 22, breeding the S44 and using somatic transfection to insert the T710 allele, a PKN2-expressing transgenic line, and using non-steroidal anti-inflammatory drugs to reduce the inflammation. These approaches, along with the use of other animal models, can be utilized to better understand the pathogenesis of glaucoma. In particular, the use of inbred mouse models can provide valuable insights into the genetic and environmental factors that influence CEC density.

Functional Analysis
The functional analysis of these genetic loci can be performed using a variety of techniques, including in vitro and in vivo assays. In vitro assays can be used to identify the specific genes and pathways that are involved in CEC density, while in vivo assays can be used to determine the physiological effects of these genes and pathways on CEC density.

Transgenic Mouse Models
Transgenic mouse models can be used to validate the genetic loci identified in the functional analysis. These models can be generated by inserting the identified genes into the mouse genome and then analyzing the resulting phenotype. This can provide important information about the role of these genes in controlling physiological functions.
Suggested structure for Approach section

**Failed Studies**

My previous studies will provide many answers about the involvement of MCU in SAM physiology and dysfunction. It is undeniable that many more questions will arise after completion of the existing body of work.

I have already made mutant MCU constructs that are likely resistant to CaMKII phosphorylation. While outside of the scope of my proposal, these constructs will help me study the relationship between CaMKII and calcium MCU in the future. I plan to learn the patch-clamping technique before finishing my project because this may be an important technique for my future studies and Dr. Anderson’s laboratory has many experts in the technique of patch clamping. I am excited and motivated to complete the work outlined in my proposal. Completing this body of work will provide important information about cardiac mitochondria and will allow me to learn basic scientific principles on which I can build an independent scientific career.

- My studies will provide answers to...
- ...many additional questions will arise.
- While outside of the scope of my proposal, these xxx will...
- In future...
- Completion of this work will provide important information about...
- ...and will allow me to learn xxx on which I can build an independent scientific career.

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**Principal Investigator/Program Director (last, first, middle, Prefix, City, State, ZIP)**

Neutralization using standard methods (24). The antibodies to MCU can be directly measured by the anti-MUCU-20, as previously demonstrated in fibroblasts (24, 25). An additional variant of this process can be used to detect the anti-MUCU-20 affinity of the antibody. We have already established the in vitro abilities of the antibody to neutralize the antibody affinity for MCU (25). The anti-MUCU-20 affinity for MCU does not vary significantly in the species. We would therefore use the same method to confirm the results in the species of interest. The antibody affinity for MCU will be determined using the anti-MUCU-20 affinity of the antibody.

**Expected outcomes and potential problems and their solutions or alternative approaches.**

These studies will use site-directed antibodies and neutralize certain situations to answer the important questions about the mechanisms of antibody attachment, recognition of specific epitopes, and downstream effects of antibody antibodies. Antibodies will be used in the experiments to confirm the results in the species of interest. The antibody affinity for MCU will be determined using the anti-MUCU-20 affinity of the antibody.

**Debrief/Summarize and Conclude.**

These studies will merge our understanding of the structure of MCU with a more detailed knowledge of the binding properties and functions of MCU and its interactions with other proteins. The results will be correlated to the antibody affinity for MCU and downstream effects of antibody antibodies. Antibodies will be used in the experiments to confirm the results in the species of interest. The antibody affinity for MCU will be determined using the anti-MUCU-20 affinity of the antibody.

**Threading.**

This project would take 3 years to complete. The sequence of studies will include in vitro and in vivo work on the use of specific and non-specific binding sites determined in the previous studies. The antibody affinity for MCU will be determined using the anti-MUCU-20 affinity of the antibody. The antibody affinity for MCU will be determined using the anti-MUCU-20 affinity of the antibody.

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