Pre-doctoral Grant Programs

1. NSF Graduate Research Fellowships
2. NSF Doctoral Dissertation Improvement Grants
4. Faculty Grants

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Grant-Winners Seminars
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1. NSF Graduate Research Fellowships

- Awarded to outstanding students in all NSF-supported STEM disciplines pursuing graduate degrees who have not yet received a master's degree
- Eligibility: US citizens, nationals, or permanent residents w/no more than 12 months graduate study
  Level 1: Prior to entering graduate school
  Level 2: 1st year of graduate school
  Level 3: Prior to completing 1st term of 2nd year
  Level 4: Significant change of field or lengthy interruption
- Awards: 3 years of support usable over 5 year period
  $32,000 stipend per year
  $12,000 cost of education allowance per year
  $1,000 one time international travel allowance
  ~ 2,700 awards/yr (~17% success rate)

Home page:
http://www.nsfgrfp.org/
Lots of good info!
GRFP Program Office

Program Announcement

Graduate Research Fellowship Program (GRFP)

PROGRAM IDENTIFICATION
NSF 14-590
SPARC 10-016

Rules for success:
1. Study this document!
2. Study this document!
3. Study this document!
4. Contact program office with questions
5. Watch for new announcement (Aug/Sept)
GRF: Key Parts of the Application:

- Personal Profile
- Education & Work Experience
- Planned Graduate Program
- Personal Statement, Relevant Background and Future Goals (3 pp.)*
- Graduate Research (2 pp.)*
- Official Academic Transcripts
- GRE scores (optional)
- 3 Letters of Reference

*Personal essays

GRF Application Statements

Two VERY IMPORTANT essays:

- Personal Statement, Relevant Background, and Future Goals
- Graduate Research (proposed plan)

Note:
Excellent manual with many examples:
"Writing Personal Statements Online"
www.e-education.psu.edu/writingpersonalstatementsonline/
Personal Statement:

- Describe any personal, professional, or educational experiences or situations that have prepared you or contributed to your desire to pursue advanced study in science, technology, mathematics, or engineering.

- Describe your competencies and evidence of leadership potential.

- Discuss your career aspirations and how the NSF fellowship will enable you to achieve your goals.

Personal Statement, cont’d:

Describe your experiences in the following, or describe how you would address the following in your professional career:

- Integrating research & education, e.g., participating in & encouraging discovery at various levels.

- Advancing diversity in science, e.g., contributing to the participation of women and/or underrepresented minorities.

- Enhancing scientific & technical understanding, e.g., sharing scientific knowledge with the general community; or

- Benefiting society.

Note: Provide specific details that address the 2 NSF Review Criteria: Intellectual Merit and Broader Impacts.
Proposed Plan of Research:

In a clear, concise, and original statement, present a complete plan for a research project that you may pursue while on fellowship tenure & how you became interested in the topic. Your statement should:

- Present your plan with a clear hypothesis (or questions) to be asked by the research
- Demonstrate your understanding of research design & methodology; explain the relationship to your previous research, if any
- Describe how you propose to address the 2 NSF Merit Review Criteria

Format:
Title/Key Words/Hypothesis (or Research Questions)/
Research Plan/Anticipated Results (or Findings)/
Literature Citations/Statement Attesting to Originality

Letters of Recommendation (3)

- Get them from Ph.D.'s who LOVE you!
- Letters from business/industry tend to be less targeted and shorter
- Give your references a draft of your essays and/or a list items you believe they can attest to
- You might expedite the process if you suggest you can provide a draft
GRF Review Criterion: Intellectual Merit

Panelists are instructed to consider applicant’s:

- Strength of academic record
- Proposed plan of research
- Description of previous research experience
- References
- GRE General and Subject Tests scores
- Choice of institution relative to plan for graduate education
- Potential to complete research-based graduate program
- Potential to produce scholarly work
- Potential to meet the scientific and technological workforce need
- Ability to interpret and communicate research findings
- Potential to work in interdisciplinary teams

GRF Review Criterion: Broader Impacts

Evidence that applicant will:

- Effectively integrate research and education at all levels
- Infuse learning with the excitement of discovery
- Assure that findings & methods of research are communicated effectively
- Encourage diversity, broaden opportunities and enable full participation
- Enhance scientific and technical understanding
- Accomplish work that will benefit society

Examples:
Electronic submission required:

FastLane
www.fastlane.nsf.gov

https://www.fastlane.nsf.gov/fastlane.jsp
Click on "DEMONSTRATION SITE"
and "FASTLANE FAQ's"

OR

GRANTS.GOV™

Hint: Use FastLane!

Very Helpful Web Page!

www.nsfgrfp.org/
2. Doctoral Dissertation Improvement Grants

- Awarded by two NSF Directorates: SBE and Biological Sciences
- Goal: Improve the quality of dissertation research

- Eligibility: Doctoral students who have finished coursework; must be enrolled in US institution; need not be US citizen

- Use of funds: Expenses for significant data gathering projects or field research in off campus settings not otherwise possible

- Awards: Up to $12 – $15,000; usually span a 24-month period (wide variation among programs)

- Dissertation advisor submits proposal on behalf of graduate student who is ready to start or already conducting dissertation research

- The Faculty member is listed as the "PI/PD" and the graduate student is listed as the "CO-PI/PD"

- Proposals are judged on the basis of scientific merit – the importance of the research and strength of the research design

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DDIG/DDRIG Program Offices

Note: Program solicitation documents for DDRIGs are now discipline specific! (see sample)

www.nsf.gov/funding/pgm_summ.jsp?pims_id=5234

www.nsf.gov/funding/pgm_summ.jsp?pims_id=13453
Study These Documents!

** Doctoral Dissertation Improvement Grants in the Behavioral and Biological Sciences (DDIG)**

**Program Solicitation**

**Important Information and Revision Notes**

**Summary of Program Requirements**

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**Cultural Anthropology Program - Doctoral Dissertation Research Improvement Grants (CA-DEDRG)**

**Program Solicitation**

**Important Information and Revision Notes**

**Summary of Program Requirements**

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GPG: The source of all truth and wisdom...

*Note: The GPG is frequently updated; be sure to use current edition!*

*Note: These are commandments, not suggestions!"
Key Parts of the Proposal:

- Project Summary
- Project Description
- References Cited
- Biographical Sketches
- Budget
- Budget Narrative
- Facilities, Equipment & Other Resources

Project Summary

- A one page advertisement; the single most important part of your proposal
- Must be a stand alone, self-contained description of the project's purpose, scope, methods and expected outcomes
- Written in accessible language and understandable to a scientifically or technically literate lay reader
- Must clearly address in separate statements:
  1) the intellectual merit of the proposed activity;
  2) the broader impacts resulting from the proposed activity
**Intellectual Merit – 5 strands**

1) How important is the proposed activity to **advancing knowledge and understanding** within its own field or across different fields?

2) How **well qualified** is the proposer to conduct the project?

3) To what extent does the proposed activity explore **creative, original, or POTENTIALLY TRANSFORMATIVE CONCEPTS**?

4) How **well conceived and organized** is the proposed activity?

5) Is there sufficient **access to necessary resources**?

*"potentially transformative" is a new emphasis (Sept. 2007)*


**Broader Impacts – 5 strands**

1) How well does the activity advance discovery and understanding while **promoting teaching, training and learning**?

2) How well does the proposed activity **broaden the participation of women and underrepresented groups**? ("Diversity")

3) To what extent will it **enhance the infrastructure for research and education**, such as facilities, instrumentation, networks and partnerships?

4) Will the results be **disseminated broadly to enhance scientific and technological understanding**?

5) What may be the **benefits** of the proposed activity to **society**?

Examples:
Project Description

- Stress the **scientific significance** of the work
- Describe a **specific research plan**: activities & methods
- Summarize **progress** to date if the research is already underway
- Include a **Research Schedule** that indicates a timeline for completion of tasks and the date funds are required
- **Page-lengths** can vary widely (8 – 15 pp.); *always consult program website or contact the program director*

Sample Project Description Outline

I. Statement of the Problem

II. Background
   A. Literature Review
   B. Preliminary Research

III. Research Objectives/Theoretical Framework
   A. Hypotheses
   B. Specific Research Goals

IV. Research Plan
   A. Research Site
   B. Research Schedule
Sample Outline (cont’d)

IV. Research Plan, cont’d
   C. Data Collection
      i. Sampling (Phase I)
      ii. Measurement (Phase II)
      iii. Surveys/Interviews (Phase III, etc.)
   D. Data Analysis
      i. Data entry and coding
      ii. Statistics

V. Significance of Proposed Research
   A. Intellectual Merit
   B. Broader Impacts
   C. Integration of Research & Education

VI. References Cited


- Awarded to outstanding graduate students in most science and engineering disciplines who intend to pursue doctoral degrees

- Eligibility: US citizens or nationals (not permanent residents)
  - Final year of undergraduate study
  - Within first 2 years of graduate study

- Awards: Up to 3 years of support:
  - Tuition & fees
  - Stipend: $30,500/ $31,000/ $31,500
  - Health Insurance up to $1,000/yr

- Deadline: December 12, 2014

- Proposal success rate: ~10%
Tips for Success...

- Be persuasive in presenting the **significance** of your research—why it will make an important contribution to the field.
- Stress **originality** of your approach.
- Develop a clearly stated and logical **research plan**, with specific, measurable **objectives**.
- Include a **time schedule** that addresses each objective.
- PI must show student will receive necessary **guidance** and **mentoring** to be successful.
- Indicate how DDIG award will lead to a **product**—book, article, or demonstrable scholarly advance.
4. Alternative Fellowship Programs

Web pages:

- COS/Pivot [http://pivot.cos.com/](http://pivot.cos.com/)
- Vanderbilt U: [www.vanderbilt.edu/psychological_sciences/graduate/current/funding.php](http://www.vanderbilt.edu/psychological_sciences/graduate/current/funding.php)
4. Faculty Grants

1. Faculty researchers frequently include graduate research assistants (GRA's) in their grant proposals
2. Departments will often post openings
3. Candidates are sometimes identified beforehand

Hint:
Be proactive, inquire about future openings!

Remember...

"The early bird may get the worm, but God does not throw it in the nest."
- P. D. James
Merit Review Broader Impacts Criterion: Representative Activities

Proposals submitted to the National Science Foundation are evaluated through use of two merit review criteria, which all proposals must address explicitly. Experience shows that while most proposers have little difficulty responding to the criterion relating to intellectual merit, many proposers have difficulty understanding how to frame the broader impacts of the activities they propose to undertake.

The examples provided below are organized by the set of potential considerations used in assessing the broader impacts of the proposed activity. They illustrate activities that, when successfully incorporated in a project description, will help reviewers and NSF program staff address the broader impacts criterion in the review and decision process.

The list is not intended to be exhaustive, nor is any particular example relevant to all proposals. Proposers can draw from the examples but are urged to be creative in their approaches to demonstrating the broader impacts of their projects. Proposers already undertaking similar kinds of activities should carefully consider how to link these examples to the research and education projects they are proposing for funding. Proposers also should consider what types of activities best suit their interests, while enhancing the broader impacts of the project being proposed.

The components of the broader impacts criterion as defined by the National Science Board are listed below. The list is followed by short sections on each component that provide background information and representative activities.

Broader Impacts Criterion: What are the broader impacts of the proposed activity?

- How well does the activity advance discovery and understanding while promoting teaching, training and learning?
- How well does the proposed activity broaden the participation of underrepresented groups (e.g., gender, ethnicity, disability, geographic, etc.)?
- To what extent will it enhance the infrastructure for research and education, such as facilities, instrumentation, networks and partnerships?
- Will the results be disseminated broadly to enhance scientific and technological understanding?
- What may be the benefits of the proposed activity to society?
Advance Discovery and Understanding While Promoting Teaching, Training and Learning

Background:

Integration of research and education is one of "three core strategies that guide [NSF] in establishing priorities, identifying opportunities, and designing new programs and activities.... Effective integration of research and education at all levels infuses learning with the excitement of discovery and assures that the findings and methods of research are quickly and effectively communicated in a broader context and to a larger audience" (NSF GPRA Strategic Plan 2001 - 2006)

Examples of Activities:

- Integrate research activities into the teaching of science, math and engineering at all educational levels (e.g., K-12, undergraduate science majors, non-science majors, and graduate students).
- Include students (e.g., K-12, undergraduate science majors, non-science majors, and/or graduate students) as participants in the proposed activities as appropriate.
- Participate in the recruitment, training, and/or professional development of K-12 science and math teachers.
- Develop research-based educational materials or contribute to databases useful in teaching (e.g., K-16 digital library).
- Partner with researchers and educators to develop effective means of incorporating research into learning and education.
- Encourage student participation at meetings and activities of professional societies.
- Establish special mentoring programs for high school students, undergraduates, graduate students, and technicians conducting research.
- Involve graduate and post-doctoral researchers in undergraduate teaching activities.
- Develop, adopt, adapt or disseminate effective models and pedagogic approaches to science, mathematics and engineering teaching.

Broaden Participation of Underrepresented Groups

Background:

One of NSF’s five-year strategies is to "broaden participation and enhance diversity in NSF programs. At present, several groups, including underrepresented minorities, women, certain types of academic institutions, and some geographic areas are less than full participants in the science and engineering enterprise. NSF is committed to leading the way to an enterprise that fully captures the strength of America’s diversity." (NSF GPRA Strategic Plan 2001-2006)
Examples of Activities:

- Establish research and education collaborations with students and/or faculty who are members of underrepresented groups.
- Include students from underrepresented groups as participants in the proposed research and education activities.
- Establish research and education collaborations with students and faculty from non-Ph.D.-granting institutions and those serving underrepresented groups.
- Make campus visits and presentations at institutions that serve underrepresented groups.
- Establish research and education collaborations with faculty and students at community colleges, colleges for women, undergraduate institutions, and EPSCoR institutions.
- Mentor early-career scientists and engineers from underrepresented groups who are submitting NSF proposals.
- Participate in developing new approaches (e.g., use of information technology and connectivity) to engage underserved individuals, groups, and communities in science and engineering.
- Participate in conferences, workshops and field activities where diversity is a priority.

Enhance Infrastructure for Research and Education

Background:

The NSF Act of 1950 authorizes and directs the Foundation "to foster and support the development and use of computer and other scientific and engineering methods and technologies, primarily for research and education in the sciences and engineering;..."

"NSF investments provide state-of-the-art tools for research and education, such as instrumentation and equipment, multi-user facilities, ... telescopes, research vessels and aircraft, ... Internet-based and distributed user facilities, ... research networks, digital libraries and large databases." (NSF GPRA Strategic Plan 2001-2006)

Examples of Activities:

- Identify and establish collaborations between disciplines and institutions, among the U.S. academic institutions, industry and government and with international partners.
- Stimulate and support the development and dissemination of next-generation instrumentation, multi-user facilities, and other shared research and education platforms.
- Maintain, operate and modernize shared research and education infrastructure, including facilities and science and technology centers and engineering research centers.
• Upgrade the computation and computing infrastructure, including advanced computing resources and new types of information tools (e.g., large databases, networks and associated systems, and digital libraries).
• Develop activities that ensure that multi-user facilities are sites of research and mentoring for large numbers of science and engineering students.

**Broad Dissemination to Enhance Scientific and Technological Understanding**

**Background:**

"NSF advocates and encourages open scientific communication. NSF expects significant findings from supported research and educational activities to be promptly submitted for publication.... It expects PIs to share with other researchers, at no more than incremental cost and within a reasonable time, the data, samples, physical collections and other supporting materials created or gathered in the course of the work. It also encourages grantees to share software and inventions . . . and otherwise to make the innovations ... widely useful and usable." (GPG; NSF 01-2a)

**Examples of Activities:**

• Partner with museums, nature centers, science centers, and similar institutions to develop exhibits in science, math, and engineering.
• Involve the public or industry, where possible, in research and education activities.
• Give science and engineering presentations to the broader community (e.g., at museums and libraries, on radio shows, and in other such venues.).
• Make data available in a timely manner by means of databases, digital libraries, or other venues such as CD-ROMs.
• Publish in diverse media (e.g., non-technical literature, and websites, CD-ROMs, press kits) to reach broad audiences.
• Present research and education results in formats useful to policy-makers, members of Congress, industry, and broad audiences.
• Participate in multi- and interdisciplinary conferences, workshops, and research activities.
• Integrate research with education activities in order to communicate in a broader context.

**Benefits to Society**

**Background:**

NSF is committed to fostering connections between discoveries and their use in service to society. The knowledge provided by NSF-funded projects offers a rich foundation for its broad and useful application. For example, projects may contribute to understanding the environment, commercial technology, public policy, health or
safety and other aspects of the public welfare. (NSF GPRA Strategic Plan 2001-2006)

Examples of Activities:

- Demonstrate the linkage between discovery and societal benefit by providing specific examples and explanations regarding the potential application of research and education results.
- Partner with academic scientists, staff at federal agencies and with the private sector on both technological and scientific projects to integrate research into broader programs and activities of national interest.
- Analyze, interpret, and synthesize research and education results in formats understandable and useful for non-scientists.
- Provide information for policy formulation by Federal, State or local agencies.
NSF Graduate Research Fellowship Application
Rebecca Wilson

- Personal Statement
- Previous Research Experience
- Research Plan
- Reviewers’ Comments
Personal Statement Essay

The entire scientific process of designing experiments, collecting and interpreting data, and even publishing the results is, in my opinion, a combination of strategy games and logic puzzles, two of my favorite activities. I enjoyed participating in these aspects of the scientific process when I worked as technician in Dr. Biao He’s lab at the Pennsylvania State University (PSU). In the process, I learned that one of my strengths as a researcher was evaluating experiments as if they were logic puzzles. For example, one time, I was given explicit instructions to do an experiment that was needed to satisfy a reviewer, which a lab member had given up on. Before I did the experiment I tried to figure out why it wasn’t working and realized that it would be impossible to obtain the desired information from this experiment. So, when I did the experiment, I added a control that no one had thought of to prove my point. Using the control, I was able to convincingly explain why the experiment would not work and I suggested an alternative experiment, which satisfied the reviewer.

Although I enjoy the process of science, the broader impacts of the research are very important to me and as a technician I felt extremely limited in this direction. Hoping to do research with a greater impact, I joined Dr. Blake Peterson’s synthetic chemistry lab at PSU as a technician and worked on a project aimed at rationally designing a compound with selective, antagonistic anti-androgen receptor activity (the androgen receptor is major drug target for prostate cancer). Working on this high risk, narrowly focused project, made me aware of how important basic research is in fueling higher impact research and that it is often impossible to solve huge problems in a short period of time. This reinforced what I learned in Dr. He’s lab that research requires patience and persistence.

I developed both of these qualities in high school and college running cross-country and distance events in track and field. In high school, I woke up at 5 am and went to the gym before school to lift weights and run and then worked out with my sports team after school. At big races where the runners had assigned starting positions, the judges would always double check that they read the correct name when I stepped up to the line because I do not look like a distance runner. But, my hard work paid off. In high school, I qualified to run in three different state track meets and one cross-country meet. In college, I ran in one Division III national cross-country meet and, in track and field, even though I look too short to jump over a steeple, I set the record at my college and ran the event fast enough to provisionally qualify for nationals. Participating in these sports also taught me that hard work and discipline are just as important as natural abilities.

While I was working in Dr. He’s and Dr. Peterson’s lab, I realized that if I wanted to pursue my own research ideas, I needed to improve my scientific knowledge in a graduate program. But, applying to graduate school was something I had never considered. I grew up in a small town with one traffic light and no McDonalds. Neither of my parents holds a bachelors degree, but I was very motivated to attend a college with a good science program. My first exposure to molecular biology was in high school. My school didn’t have a lot of resources, but my biology teacher received a grant to buy a pipette and an electrophoresis chamber. My class was the first to use it and I still remember borrowing gloves from the nurses’ office and a microwave from the teachers’ lounge to make the agarose gel. I was amazed by this little gel electrophoresis experiment and it played a major role in my decision to major in the sciences even though I was clueless about what scientists actually did and had no idea what I would do when I graduated.
As a technician and because my husband was a graduate student in chemistry, I learned about graduate school. When my husband graduated from PSU with a Ph.D., we moved to Knoxville, Tennessee for his job and I applied to the graduate program in the Department of Biochemistry, Cellular and Molecular Biology at the University of Tennessee. During my first year as a graduate student, I did research rotations in three different labs, Dr. Elena Shpak, Dr. Albrecht Von Arnim (plant molecular biology), and Dr. Dan Roberts (plant biochemistry), but I was so excited about the role of EKI1, an E3 ubiquitin ligase, in ERECTA signaling, that I decided to stay in Dr. Shpak’s lab to do my Ph.D. research. Her lab studies plant growth and development using Arabidopsis as the model system and, specifically, studies ERECTA, a leucine-rich repeat (LRR) receptor-like kinase (RLK), that is involved in regulation of aboveground organ growth. I worked on a project to functionally characterize and test whether two proteins found to interact with the intracellular domain of ERECTA in a yeast two-hybrid library screen, EKI1 and AraP62, are part of ERECTA mediated signaling. I discovered that EKI1 positively regulates ERECTA signaling by stabilizing ERECTA. My research proposal focuses on determining the mechanism of EKI1-dependent ERECTA stabilization, but this is only one of my exciting projects. I am also examining the role of AraP62 in ERECTA signaling and working on a project to analyze ERECTA autophosphorylation, which I started during my rotation in Dr. Roberts’ lab.

One of my most rewarding experiences as a technician was mentoring undergraduate students, so I am continuing this activity. While working in Dr. Shpak’s lab, I have mentored one undergraduate student and three high school students. I also participated in the Tennessee Science Olympiad for middle school students by helping in the “bio-processing” part of the competition. The high school students do research in our lab through the Upward Bound Regional Center Mentoring program at the University of Tennessee (funded by the US Department of Education) that was created to provide research opportunities for high school students from low-income families where neither parent has a bachelor’s degree. I mentored Lasasha Whitfield (African American female) one summer and Hanna Veit (female) and Zachary Hall the next. Under my guidance Zach and Hanna each created a plasmid, which will be used to examine the ability of EKI1 family members to stabilize ERECTA. They also made a poster, gave a presentation, and wrote a paper about their project, which was part of a competition that all of the students in the program competed in. Zach and Hanna’s project was very technical, but they did such a good job explaining their project that by the end of the competition, the judges, with no science background, were excited about their project and they received second place in the research competition. Zach Hall chose to continue working in our lab. In addition to getting these students excited about science, I hope I was a positive role model for them and can continue to work with under-represented undergraduate and high school students.

I am motivated to learn as much as possible while pursing a Ph.D., so I will be well equipped to tackle my own research ideas. I am particularly interested in the ability of differentiated cells to dedifferentiate and become stems cells. In the future, I would like to be a principle investigator of a large, well-funded lab and address the following questions: What is the mechanism of cellular dedifferentiation that allows plants to form new stem cells? What regulatory mechanisms control this process and prevent cancerous growth? Why aren’t animal cells able control dedifferentiation to heal damaged tissue or to prevent cancer? This NSF grant will support my graduate education and provide me with the training to become a successful scientist.
Previous Research Experience

My first scientific research experience, as a technician in Dr. Biao He’s lab at the Pennsylvania State University (PSU), has impacted my view of scientific research and given me the basic skills I need to pursue an advanced degree in science. In Dr. He’s lab, despite having no undergraduate research experience, I learned the techniques quickly and was given my own project: to determine the function of the small hydrophobic (SH) protein in mumps virus (MuV) strain Enders. The research I did is significant for two reasons. First, it indicated that small hydrophobic proteins may be functional homologs and prevent apoptosis by inhibiting tumor necrosis factor (TNF)-α induced, NFκB activated, gene expression regardless of the SH protein sequence similarity. Secondly, it established a reporter gene assay that can be used to quickly determine if other SH proteins are able to inhibit TNF-α induced, NFκB activated, gene expression without generating a recombinant virus, which is not always possible.

Optimizing the reporter gene assay, turned out to be the most exciting and frustrating aspect of my project. In this assay, done in mammalian tissue culture cells, expression of a firefly luciferase reporter gene, which is under control of an NFκB dependent promoter, is induced by addition of exogenous TNF-α. The assay examines the ability of SH to inhibit expression of the reporter gene. Dr. He designed the assay a couple of years before I joined the lab to study simian virus 5 SH function, but never published the results. My task seemed very simple: repeat the experiment with MuV SH. However, close to a year later, I was still not able to reproduce the original results. Meanwhile, the experiments that I was told were “technically challenging,” such as an electrophoretic mobility shift assay (EMSA), were giving me beautiful and repeatable results. EMSAs have a number of applications, but all involve examining protein/DNA interactions. I isolated nuclear localized proteins from tissue culture cells that were infected with either wild-type or recombinant virus and used the EMSA to determine if active NFκB transcription factor was localized in the nuclear fraction.

After trying to optimize the reporter gene assay for about half of a year, we finally realized the negative control was problematic, but could not figure out the reason it had such a high background level of reporter gene expression. Then one day, when I was ready to test my newest hypothesis concerning the negative control, we ran out of the transfection reagent I needed. I was too excited and impatient to wait another week to start my experiment, so I borrowed a similar reagent from another lab. To my surprise, the experiment worked, but my hypothesis was obviously not correct. It appeared that the new transfection reagent I used lowered the background of the negative control. I repeated the experiment to compare the transfection reagent used in my lab with the one I had borrowed from the other lab and determined that our transfection reagent was inducing a response in my negative control. This experiment was extremely frustrating because for almost an entire year, I felt like I was making a really stupid mistake and my frustration was compounded by the fact that I could not figure out what I was doing wrong. Even though I ended up solving the problem because of pure luck, which I find somewhat unsatisfying, I loved thinking about the data and it was very satisfying to finally solve the problem.

One of the most important things I learned about while working on this project, which I had never even considered before, is the ethical aspect of science. I repeated the above experiment so many times that I felt like it was hoped I would give up on troubleshooting the experiment and fabricate my data to obtain the “expected” results. In the end, I solved the problem, but this situation made me aware of how important it is to repeat an experiment more
than once even if I get the expected results the first time. I also learned that it is ideal to do more than one type of experiment to test my hypothesis and to carefully interpret even what seem to be the simplest results, keeping in mind that my original hypothesis may not be correct.

The most rewarding experience I had in Dr. He's lab was mentoring the undergraduate students. It started because I noticed that Elizabeth Cohen (Jewish American female) seemed very frustrated and discouraged with her project to clone a viral gene. So, everyday she came to lab, I would ask her what she was doing and then re-explain it to her in a way that she could understand and wasn't just repeating directions. Then I would help her with any problem solving she needed to do. As a result, when the next undergraduate student, Erica Taddeo (female) joined our lab, she was assigned to me from the start, as were Nikki Ford and Mercedes Stoneberg (both female). I worked on a different project with each of the students. My love for any type of experiment that involves microscopy is contagious, so it was easy to get Erica excited about my project. One of the experiments I taught her was an immunofluorescence assay, which we used to examine NFκB localization. For this experiment, we infected mammalian tissue culture cells with wt or recombinant virus, fixed the cells to cover slips, and incubated them first with primary antibody against NFκB and then with a secondary antibody containing a fluorescent tag. We examined the localization of NFκB using a fluorescent microscope. Erica did excellent work and was an author on my paper. It was really exciting to be part of her science education and rewarding when I heard that she received a well-paying position in industry after she graduated.

I had the opportunity to present the results of my research to the scientific community at the annual American Society for Virology meeting in 2005 and then to publish the results in 2006 in the Journal of Virology. Dr. He afforded me the unique opportunity to write the first draft of the paper and involved me in all of the additional drafts. This was a great way to finish my project. I was amazed at how much I was able to learn and then teach the undergraduate students in such a short time.

Overall, my experience working as a technician was rewarding. I have also done research as a technician in two additional labs, Dr. Blake Peterson's lab also at PSU and Dr. Elena Shpak at the University of Tennessee. In these labs I gained a broad range of skills encompassing both biochemistry and genetics techniques. Through these experiences, I realized that I possess many qualities that will make me a successful scientist, such as determination, good ethics, a little luck, and a contagious excitement about my research that I hope continues to have a positive impact on the undergraduate students I mentor.

Publications and presentations:
Receptor-Like Kinase Stabilization in Arabidopsis Growth and Development

Key words: ERECTA, growth and development, receptor-like kinase

Background: Plants develop post-embryonically by the continual activity of a population of stem cells in the apical meristem. While the meristem determines the size, number and identity of organ primordia, later processes determine the final size and shape of the organ. In Arabidopsis, ERECTA family genes (ERECTA, ERL1 and ERL2 referred to as ERLs) have been proposed to synergistically promote coordinated cell proliferation in aboveground organs. Arabidopsis plants that lack ERECTA are shorter in height and have shorter pedicel and silique lengths than wild type (wt) due to reduced cell numbers. In addition to growth defects, the erecta/erl1/erl2 mutant exhibits stomatal clustering and an increased stomatal index [1, 2].

ERLs are receptor-like serine/threonine kinases located in the cell membrane via single transmembrane domains [3]. Arabidopsis contains over 600 putative serine/threonine receptor-like kinases (RLKs), that have a variety of functions, including pathogen resistance, growth and development, and self-incompatibility [4]. ERECTA is a member of the largest family of RLKs that are distinguished by their extracellular leucine-rich repeat (LRR) domain. Several proteins are proposed to be part of ERECTA signaling based on genetic and phenotypic analysis [2, 5, 6].

ERECTA and ERECTA-like genes are found in all sequenced vascular plant genomes. Exciting data in a DuPont patent suggests that overexpression of ZmERECTA in maize results in a reduced stomatal index that may improve their drought resistancetance [7]. Other potential benefits of ERECTA overexpression include increasing the biomass of plants grown for fuel or the fruit size of crop plants. Decreasing ERECTA may prove beneficial to grains that are prone to wind lodging. Additional understanding of ERECTA signaling and regulation, such as that proposed below, may allow for fine-tuned manipulation of ERECTA signaling in crop plants.

Preliminary data: In order to identify novel components of ERECTA signaling, a yeast two-hybrid (Y2H) library screen was conducted with the juxtamembrane and kinase domain of ERECTA as the bait and a cDNA library from Arabidopsis seedlings as the prey. EKIP1 (for ERECTA kinase interacting protein 1) was chosen for further analysis. It belongs to a family of 4 proteins, which contain an N-terminal RING-H2 finger domain, characteristic of E3 ubiquitin ligases [8] and a conserved C-terminus that interacts with ERECTA in a Y2H assay. Preliminary data obtained with a promoter beta-glucuronidase (EKP1::GUS) assay indicates that EKIP1 is expressed in young tissues where ERECTA is thought to function. A ubiquitination assay shows that EKIP1 has E3 ubiquitin ligase activity. In order to investigate the function of EKIP family genes in vivo, Arabidopsis T-DNA insertion lines available from Arabidopsis Biological Resource Center and EKIP1 RNAi lines were generated and analyzed. No reduction in EKIP1 expression was observed in these lines; therefore, to study EKIP1 function, transgenic Arabidopsis were generated in which EKIP1 is expressed under control of the ERECTA promoter (ER::EKIP1). Three independent ER::EKIP1 lines, in which EKIP1 is thought to be co-suppressed, produced plants with an intermediate erecta phenotype.

Because E3 ubiquitin ligases can target proteins for degradation, the effect of EKIP1 expression on ERECTA protein levels was examined. ERECTA is a very low abundance protein; therefore, in order to facilitate its detection, ERECTA was translationally fused to renilla luciferase and expressed in erecta plants with the ERECTA promoter (ER-RLUC). Three independent lines of ER::EKIP1 co-suppression lines were crossed with two independent lines of ER-RLUC. RLUC activity was examined in F1 plants homozygous for both ER-RLUC and ER::EKIP1, and plants homozygous for ER-RLUC, but lacking ER::EKIP1. ER-RLUC
expression was ten times less in plants expressing ER::EKIP1 (despite no change in ERECTA expression). Transient overexpression of EKIP1 in Arabidopsis seedlings leads to a two-fold increase in ERECTA-RLUC expression. This complementary data suggests that EKIP1 may function to stabilize ERECTA. My working hypothesis is that EKIP1 positively regulates ERECTA signaling by stabilizing ERECTA. The specific aim of this proposal is to determine the mechanism of EKIP1-dependent ERECTA stabilization.

Specific aim 1: Test whether EKIP1-dependent ubiquitination is required for ERECTA stabilization. EKIP1 has E3 ubiquitin ligase activity, however this activity may not be required for ERECTA stabilization. An alternative hypothesis is that EKIP1 stabilizes ERECTA by interacting in a way that “hides” a proteolytic site (unpublished data). In order to determine if E3 activity is required for EKIP1-dependent ERECTA stabilization, the effect of transiently overexpressing EKIP1 or an EKIP1 mutant lacking E3 activity (EKIP1m) on ERECTA-RLUC will be examined in Arabidopsis seedlings using a luciferase assay. EKIP1m will be made by mutation of the conserved cystine residue required for E3 activity and its activity will be tested with a ubiquitination assay. If EKIP1 E3 activity is required for ERECTA stabilization, overexpression of EKIP1m should not affect ERECTA-RLUC expression.

Specific aim 2: Determine EKIP1 substrate(s). It is possible that EKIP1 stabilizes ERECTA by ubiquitinating it in a way that prevents or allows interaction with a negative or positive regulator, respectively. A ubiquitination assay will be done to determine if EKIP1 ubiquitinates ERECTA. If ERECTA is ubiquitinated, antibodies that specifically detect K63 (a signal for endocytosis) or K48 (a signal for proteosome degradation) polyubiquitination can be used to further elucidate the role of EKIP1-dependent ubiquitination in ERECTA stabilization and signaling. Alternatively, EKIP1 may ubiquitinate a negative regulator of ERECTA, such as and a protease, and target it for degradation. A Y2H assay using EKIP1 as the bait will be performed to identify putative substrates. If EKIP1 expression is high enough, tandem affinity purification (TAP) will be used to co-immunoprecipitate EKIP1 interaction proteins. Interesting EKIP1 interacting proteins will be genetically tested for a role in ERECTA signaling.

Conclusion: Two other receptor-like kinases, Brassica S receptor kinase (SRK) and rice XA21, are known to interact with E3 ubiquitin ligases, ARC1 and Xb3 respectively, which function as positive regulators [9, 10]. The similarities between Arabidopsis ERECTA, Brassica SRK, and rice Xa21 kinase interaction with and positive regulation by E3 ubiquitin ligases, suggest that there may be a conserved mechanism for E3 ubiquitin ligase function in RLK signaling. Therefore, this research has the potential to extend beyond that of ERECTA signaling and to advance the scientific community’s understanding of RLK signaling. Allie Willet (undergraduate) and Zach Hall (high school student) will be working with me on this project.

References:
Review 1:
Overall Assessment of Intellectual Merit: Very Good
Explanation to the applicant: Your vast experiences in several labs has laid a sturdy foundation for a successful research career. You have indicated that you have abilities to communicate your findings, both orally and in written form (by publications and posters). Your research proposal was well written, though the hypothesis was quite vague. The preliminary data indicated that you have the tools to complete this project, and the aims were quite clear.

Overall Assessment of Broader Impacts: Excellent
Explanation to the applicant: You show a sincere desire to take science to the general public by the number of out of lab activities that you are involved in. You truly want to see the next generation of scientists succeed, and that is evident in your mentoring abilities (even including them in your thesis proposal). It is evident that you will continue this throughout graduate school, as well as further into the future. Your proposal is also relevant to society as a whole, with the potential of it benefit agricultural science.

Review 2:
Overall Assessment of Intellectual Merit: Excellent
Explanation to the applicant: Good but not exceptional academic record. Extensive prior research experience, including two first author publications in top tier journals and both a poster and a presentation at national meetings. Well-documented understanding of the importance of experimental design, including appropriate controls. Proposed research is well designed and clearly explained, and appears to consider multiple possible outcomes. More background on the role of ERLs and specifically LRR-domain proteins would have strengthened the proposal - the importance of understanding the ERECTA pathway could have been clearer. The description of the preliminary data became a bit of an alphabet soup and would have been easier to follow if summarized in a figure.

Overall Assessment of Broader Impacts: Very Good
Explanation to the applicant: Extensive experience mentoring high school students and undergraduates, and a stated intention/desire to continue doing so. Praised for her innovative teaching and her willingness to devote extra time, beyond expectations, to students she's teaching or mentoring. Application would be stronger if other aspects of Broader Impacts criterion were addressed.

Review 3:
Overall Assessment of Intellectual Merit: Excellent
Explanation to the applicant: You have shown the ability and a talent for designing and carrying out critical research. Your proposal identifies a significant problem in plant development and then outlines a series of careful experiments aimed at testing a hypothesis. Your analytical and critical skills are strong, as shown by the inclusion of proper controls and consideration of different outcomes and back-up strategies. You have demonstrated your productivity in the lab and are an author on two publications and two poster presentations.

Overall Assessment of Broader Impacts: Very Good
Explanation to the applicant: You have shown a talent for engaging and instructing disadvantaged high school students in science research. You clearly find this activity rewarding and there is amply evidence that you are very good at it, showing that you will likely be a model for integration of research and teaching.
Applying for a Postdoctoral Fellowship

1. NSF Fellowships
2. Alternative Programs
3. Faculty Grants

Robert Porter, PhD
Grant-Winners Seminars
Knoxville TN
reporter@grant-winners.com

I. NSF Postdoc Fellowships

Goal:
To provide support for career transition:

- Immediately following earned doctorate (up to 2 or 3 yrs)
- To full time professional position as independent researcher or teaching faculty
- Strong emphasis on research, but growing emphasis on teaching
- Fellowships designed for a greater leadership role than typical under a PI grant
Eligibility requirements

Proposals must be submitted by individuals who:

- Are citizens or permanent residents (green card) (Exception: NATO Partner countries—there are many!)
- Current grad student, or PH. D. for 3 yrs or less
- Present integrated research and education plans (some programs)
- Select host institution different from degree
- Not be a named participant on any current NSF proposal

Note:
Eligible disciplines vary from year to year—check NSF web pages!

Current NSF Postdoctoral Fellowships:

www.nsf.gov/funding/education.jsp?org=DRL&fund_type=3
Stipend and Allowances
(Note: Varies by program!)

EX: Earth Sciences (EAR-PF)

- Period of support: 2 years
- Total budget: $87,000/yr
  - Stipends: $62,000
  - Research, office, travel, insurance: $25,000

Note: No other appointment or remuneration may be accepted from any source!

Writing your proposal

1. NSF Cover Page
2. Project Summary (1 page)
   - Host institution
   - Sponsoring scientist(s)
   - Overview
   - Intellectual Merit
   - Broader Impacts
3. Project Description (10 pp.) Details on:
   - Research and education activities
   - Justify choice of institution and sponsor(s)
   - Long term career goals & role of fellowship
4. References cited
5. Biographical sketch (2 pp. NSF format)
**Intellectual Merit – 5 strands**

1. How important is the proposed activity to **advancing knowledge and understanding** within its own field or across different fields?*

2. How **well qualified** is the proposer to conduct the project?

3. To what extent does the proposed activity explore **creative, original, or potentially transformative concepts**?

4. How **well conceived and organized** is the proposed activity?

5. Is there sufficient **access to necessary resources**?

*Strongest emphasis as of Jan. 2013; must be linked to "societal benefits" theme of Broader Impacts

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**Broader Impacts – 5 strands**

1. What may be the potential **benefits to society**?

2. How well does the activity advance discovery and understanding while **promoting teaching, training and learning**? ("Education")

3. How well does the proposed activity **broaden the participation of women and underrepresented groups**? ("Diversity")

4. To what extent will it **enhance the infrastructure for research and education**, such as facilities, instrumentation, and collaborations?

5. Will the results be **disseminated broadly** to enhance scientific and technological understanding?

*Strongest emphasis as of Jan. 2013; must be linked to "advancing knowledge" theme of Intellectual Merit

Examples:
Educational Component
(Note: Varies by program!)

EX: Earth Sciences Fellowship (EAR-PF):

- Guideline: 10% - 25% of total effort
- Examples:
  - Teaching one course each year at host institution
  - Developing educational materials (formal or informal)
  - Engaging in outreach or public education
- Strongly recommended: Work this out with host institution!

Writing your proposal, cont’d

6. Current & Pending Support (other applications)

7. Commitment letter
   - Signed by sponsoring scientist & dept. chair
   - Affirm approval of proposal
   - Commit adequate facilities & other support
   - Discuss role of sponsor as mentor & opportunities for research/training
   - If more than one host institution, same information

8. Letters of recommendation (for some programs)
GPG: The source of all truth and wisdom...

Note: The GPG is frequently updated; be sure to use current edition!

Note: These are commandments, not suggestions!

Crafting a Successful Proposal

Provide clear, concise answers to key questions:

- Why is this study important?
- Are the experiments feasible?
- What will be accomplished?
- How will it change the field?
Crafting a Successful Proposal

Design a clear experimental plan:

- Devise a concise **goal statement**, followed by 2 – 5 specific and measurable **research objectives**.
- Keep rest of proposal **focused** on this structure.
- Describe **outcomes**: What will you learn?
- Anticipate **pitfalls**: outline **alternatives**.
- Provide a **timeline**: Limit experiments to what can be accomplished within the time period.

Tips for Best Reference Letters

- Develop effective working relationships with potential referees.
- Keep your referees updated on your progress.
- Make your referees’ job easy, provide:
  - Current CV, reprints of papers
  - Draft of proposal

*Remember: This is a personal and professional relationship that may last your entire career!*
Submitting your proposal

1. Register on FastLane (not Grants.gov!)
   www.fastlane.nsf.gov/fastlane.jsp

2. Click on “Postdoctoral Fellowships and Other Programs”

3. Click on “Individual Registration”

4. After registration, go back and click on “I am an applicant”

5. Select Fellowship program and follow instructions

6. Stuck? Get help from Research Office!

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Alternative Postdoc Programs

Web pages:

- InfoED Spin Plus:  http://infoedglobal.com/
- COS/Pivot          http://pivot.cos.com/
- Vanderbilt U:      http://as.vanderbilt.edu/supportservices/grantopportunities/
Great Portal...

Fellowship and Grant Opportunities for Faculty
HUMANITIES AND SOCIAL SCIENCES
DEADLINE: DECEMBER 1, 2013 - MARCH 31, 2014
NOTE: Many deadlines are estimated deadlines; based on prior application cycle. Please confirm deadlines with granting agency.
- Alphonse D'Alby Fellowship in History, Culture, and World Politics
- Fellowship in the Social Sciences
- Fellowship, "Women, Culture, and the American Visit"
- Grants for Travel to Libraries, Archives, Collections, or Other Research Sites
- Fellowship in Romance Languages and Literatures, Humanities, and Social Sciences
- Fellowship in the Humanities and Social Sciences

Fellowship and Grant Opportunities for Graduate Students
HUMANITIES AND SOCIAL SCIENCES
http://as.vanderbilt.edu/supportservices/grantopportunities/

III. Faculty Grants

1. Faculty researchers frequently include postdoctoral appointments in their grant proposals
2. Candidates are normally identified beforehand
3. NSF now requires a "Postdoctoral Mentoring Plan" for all proposals identifying and budgeting for a postdoc

Hint: Be proactive, work with faculty member on grant proposal and Mentoring Plan!
Remember...

"The meek may inherit the earth, but not the grant dollars."
- J. Paul Getty
GUIDE TO APPLYING FOR THE NSF GRFP

What is this web site all about?

We are trying to provide information specifically geared towards physical anthropologists and evolution biologists, that will help students to put together a competitive application for the NSF GRF. It is our hope that by providing access to previous successful applications in this field you may be able to put together a strong application.

What is the NSF GRF?

The National Science Foundation (NSF) Graduate Research Fellowship Program (GRFP) offers fellowships a three-year annual stipend of $30,000 along with a $10,500 cost of education allowance for tuition and fees, a one-time $1,000 International travel allowance and the freedom to conduct their own research at any accredited U.S., or foreign institution of graduate education they choose. Basically the NSF decides to fund a researcher not a specific project, with the aim of ensuring "the vitality of the human resource base of science and engineering in the United States and reinforces its diversity." Furthermore at Stony Brook the tuition is less than the $10,500 allocated for tuition and the remainder is put into a research expense fund for the fellow.

Who can apply?

1. Must be a US citizen, US national, or permanent resident alien
2. Be in or be applying for a research-focused Master's or Ph.D. program in an NSF-supported field and have completed no more than twelve months of full-time graduate study (or the equivalent)

What do you need to apply?

1. Personal Statement Essay
2. Previous Research Experience Essay
3. Proposed Plan of Research Essay
4. 3 Reference Letters
5. Academic Transcripts
6. GRE scores - recommended, but NOT required

What are they looking for?

http://www.anat.stonybrook.edu/IDPAS/student_grants/Basicinformation2.html

9/20/2011
Applying for the NSF Predoctoral Fellowship (GRFP)

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Purpose of this guide

The primary purpose of this guide is to assemble information on the requirements for the complete NSF application in a concise and accessible format. The secondary purpose is to assist applicants in constructing a successful application. A bank of submitted applications, review sheets, and the comments of previous applicants on the process has been assembled. This resource can be used to explicitly identify the criteria used by reviewers to evaluate applications, and to benefit from the experiences of previous applicants.

Why you should apply

Winning an NSF fellowship is beneficial to you, to BCMB, and to Johns Hopkins in general. The stipend for NSF fellows is higher (by about $5000 in 2005) than the BCMB stipend. An NSF fellowship (or honorable mention) is very prestigious and is an excellent addition to your CV. The NSF funding allows BCMB to take an additional foreign student who cannot be funded by the NIH training grant, and having NSF fellows in the program enhances BCMB's reputation. Later on, the NSF funding will save your advisor the cost of your stipend—money which is then available to be spent on experiments.

Eligibility requirements
Only US citizens or permanent residents who are in the beginning stages of graduate study are eligible. There is some flexibility in the definition of "the beginning stages of graduate study;" an optional application component can be used to explain unusual circumstances. Refer to the program announcement for further information.

Due dates

The application is due November 8th. The three letters of recommendation are due Dec. 31st. Check Fastlane for confirmation.

Application components

- Personal Profile
- GRE Scores
- Transcripts
- Education and Work Experience
- Planned Graduate Program
- References
- Personal Statement
- Previous Research Experience
- Research Proposal

Personal Profile

This form collects your personal identification information.

GRE Scores

Including your GRE scores in the application is voluntary, and free of charge if you choose to do so.

Transcripts from all post-secondary educational institutions

Be sure to request transcripts using the appropriate forms, well in advance of the due date.

Education and Work Experience

The education portion of the form is a list of all colleges and universities attended and degrees awarded. The "other experience" section is where internships, fellowships, relevant summer jobs (e.g., "worked at Pfizer" but not "worked at Target") can be listed. If a notable experience helped you focus on your chosen field of study, or intensified your desire to continue in that field, it may be worth listing even if it isn't directly related to the field of study. For example, if volunteering in a public clinic in an impoverished region for spring break helped
you decide to study malaria, it may be worth including. Significant experiences that did not contribute to your advancement in your field of study should not be included.

Planned Graduate Program

Select Johns Hopkins School of Medicine as the university, and an appropriate primary field of study. Your field of study and the topic of your proposal should match; if your proposal is chemically oriented, for example, your field of study may not be in the life sciences. The BCMB program is multidisciplinary, and can be divided evenly between biochemistry, cell biology, and molecular biology. If you do not select a life science as your field of study, it will be important to explain how you intend to study outside the life sciences in the BCMB program in your personal statement or in the description of research experiences.

References

Ask your recommenders well in advance. The recommendations are submitted electronically, and are due December 31st, rather than Nov. 1st with the rest of the application.

Personal Statement

The NSF is looking for more than just science, and this is your opportunity to show your entire personality. They do want to see commitment to science, and, even more than commitment, passion. Presumably you enjoy science; now is the time to demonstrate that, as well as any of your other passions, particularly those which synergize with your scientific interests. The personal statement should not be a laundry list of your career goals and the steps to achieving them; your motivation and interest should be conveyed by the mood and tone of your writing.

The personal statement is where you make the case for the broader impacts of funding your graduate study. When discussing your goals, also discuss the broader impacts that achieving your goals will have. If you want to work on a disease-related question, describe how the world will change with increased knowledge of the disease. You should also explain how the work in your research proposal relates to your ultimate goals.

Refer to the remarks of previous applicants and the application bank for further information.

Previous Research Experience

In this section you should try to show the reviewers that you have some familiarity with the research environment and the kinds of things that you will be doing in graduate school and as a scientist. Include your most significant research experience, such as work done in a summer research fellowship, post-baccalaureate research, independent study or honors thesis projects, or even meaningful classroom laboratory projects. Explain your projects, the purpose of the research that you did, and what you learned from it. Be sure to mention any publications that may have resulted from your work on which you may be listed as an author or co-author, as well as any formal presentations, posters, seminars, or conferences in which you may have participated. You may also wish to discuss the work that you are doing in your first rotation.
Proposed Plan of Research

Successful research proposals, regardless of whether they are submitted by undergraduates, graduate students, postdocs or principle investigators, have a number of elements in common. As an applicant, your job is to frame clearly the scientific question being asked and then to convince the reviewers of three simple things: first that the question is worthwhile; second that the time needed to answer the question is reasonable in terms of the period of the grant; third that you should be funded to carry out the work.

Of these, the most important is the interest of the proposed work itself. If the question being framed is of no interest to the reviewers, then you will be fighting an uphill battle to convince them to fund you. In other words you need to capture the imagination of the reviewers and engage them in thinking about the question that interests you. This involves setting up your proposed work in the context of what has already been done in the field. If you can get the reviewers to think that yours is a great idea, then you are halfway there.

What remains then is to convince the reviewers that that the experiments are "do-able" and "do-able" by you. In other words, that the techniques are available to answer the question you have posed in the timeframe of the grant and that you have the intellectual and experimental wherewithal to get the experiments done. If you can capture the imagination of the reviewers and if after reading your application, they believe that you can actually do the work, you stand a good chance of being funded.

Try to give a roadmap to the reviewers. At every step in the proposal, the reviewers should be able to see where the proposal is coming from and where it is going. In fact in the perfect proposal, the reviewers should be already thinking of your experiments before reading that part of the proposal; then all you have to do is satisfy their expectations.

THE PARTS OF A PROPOSAL

1. CHOOSING A TOPIC

This is probably the hardest part of your proposal, because choosing a topic involves thinking through the entire proposal. Once you have settled on a topic, you will have already sketched out the kinds of experiments you are going to do and what the results of those experiments will mean one way or another to your hypothesis.

Optimally, choose an area that you are familiar with, either from undergraduate research experience, or from reading that you have already done. Your chosen area need not be the exact area you might have worked in as an undergraduate, but should be close enough that you are comfortable with the concepts and vocabulary specific to the field. Carry out a MEDLINE search in your specific area. Maybe start by reading some recent reviews with an eye to understanding the basic questions being asked in your area and what remains to be done. If you find a particular area in which you have significant questions with no apparent answers, this is a good potential topic. Perhaps you will be helped by the discussions of recent papers you find particularly interesting; there the authors may lay out the still unanswered questions or areas where their data is in conflict with earlier work. Likewise in recent reviews, the unanswered questions may be made explicit; alternately, what is not reviewed (because it hasn't been done yet) may be just as useful to you as what is reviewed. One strategy that has worked well for applicants is to imagine the "next phase" or "next question" being tackled in the lab they used to work in. Think big enough that the question is
interesting but not so big that it is impossible to tackle in one Ph.D. time unit.

Your topic needs to be focused and needs to be hypothesis driven. It may be helpful to ask yourself if your topic can be phrased in the form: "we are testing the hypothesis that x,y,and z are true.

Try to avoid choosing a question that may not have been answered in your chosen field but which has been addressed in exquisite detail in a different system. A proposal promising to clarify the mechanism of transcriptional activation in cell type A since it has been studied extensively only in cell type B will have to make the case that something is fundamentally different about A cells and B cells. Otherwise, the work will be likely only to duplicate what has been done earlier.

2. SPECIFIC AIDS/BACKGROUND

There is an important thing to remember about a reviewer. They are likely to be overworked and have many proposals to read, thus, yours is unlikely to get all the attention it deserves. You can counteract this fact by making your proposal lucid and seeing that it presents a logical progression of hypotheses and experiments. If on the other hand, the reviewers have to think too hard about what exact question you are asking, or what exactly you are doing to answer the question, they will probably simply set your proposal aside without discovering that the idea at the center is really very good.

Very early in your proposal state clearly what question you are asking. Even if your final proposal does not use it directly, write out 1 or 2 sentences that summarize the question you are asking, including the details you think most relevant. For example:

In this proposal, we aim to test the hypothesis that the polycomb group (PcG) genes in Drosophila mediate transcriptional repression by altering chromatin structure; specifically that one or more PcG gene products bind directly to nucleosomes and antagonize chromatin remodeling by the SWI/SNF complex.

Such a sentence immediately lets the reviewers know your hypothesis and more importantly stimulates him into thinking what experiments they would do to answer your question. Place it close to the beginning of your proposal. One way of organizing the beginning of your proposal is to give a brief introduction followed by the specific aims and then a more detailed background tailored to your specific aims. For example:

The Polycomb group (PcG) genes of Drosophila melanogaster have been genetically implicated in the developmentally regulated transcriptional repression of homeotic genes. These genes are thought to act by a stable modification of chromatin, although the details remain to be elucidated. In this proposal, we aim to test the hypothesis that the polycomb group (PcG) genes in Drosophila mediate transcriptional repression by altering chromatin structure; specifically that one or more PcG gene products bind directly to nucleosomes and antagonize chromatin remodeling by the SWI/SNF complex.

The PcG proteins repress transcription of a number of homeotic genes including the bithorax locus (BX). They act through a small number of cis-sequences that serve to nucleate repression of the entire region. Pc genes are genetically antagonized by mutations in the Drosophila SWI/SNF: mutations in brahma suppress Pc group mutations. This suggests that PcG genes may act by a chromatin remodeling
mechanism similar to the ATP-dependent chromatin remodeling seen from the SWI/SNF complex.

The first paragraph in a sense introduces the general area and then gives the specific question that will be addressed in the proposal. The second paragraph now introduces more background details relevant to the specific aims. Depending on the length of the proposal, this could be expanded to include, for example, additional details about SWI/SNF and other background concerning the nature of the PcG repression in Drosophila and its connection to SWI/SNF. The main point is that in these two paragraphs, you have put your project in the context of what is already known and now as the reviewers proceed they have expectations about the kinds of experiments needed to test your hypothesis.

[top]

3. METHODOLOGY

By this point in writing your proposal, you have found a topic and framed a question within the context of what has already been done. You now need to convince the reviewers that you know how to test the hypothesis you have proposed. This involves making clear that you are familiar with the technology you need to address the question, but even more importantly that you know how to interpret the results of your experiments. In this section, as well, you need to make clear that you have thought about potential weaknesses in your experimental approach and have alternative approaches in mind. You can see that for a two or three page proposal there will not be much room for experimental detail.

The difficult part of this section is knowing the level of detail to include. A common mistake is to include excessive detail about a particular protocol in order to show the reviewers that you know everything there is to know about some protocol. The effect can be the exact opposite. The danger is that the reviewers are convinced that you have read the details of a protocol but without necessarily understanding the critical elements or the principles involved. Thus, in describing the building of a construct for deletion of a gene in ES cells in mice, the last thing you want to do is include details of the restriction digests required for construction; rather you want to point out the essential parts of the construct — for example a neo gene for positive selection of the homologous recombinant and a TK gene in the vector for selection against non-targeted disruption. This shows that you have grasped the important issues for the method you are proposing to use, which is what the reviewers are really interested in seeing.

Equally importantly, it is not enough to list a set of even carefully thought out experiments that test your hypothesis. Where applicable, you need to point out what a particular result will mean to your hypothesis and to the progress of your proposal. If, for example, you suggest deleting a putative transcription factor in mice to see if it has a role in development of the forebrain, point out what complications there could be to interpretation of the phenotype and in the case that the complications preclude any useful conclusion, suggest alternative approaches you might consider.

Pay particular attention to controls for any experiments you propose. Think through the proposed experiment and try and suggest experiments that control for potential artifacts in your approach or for potential complications in interpretation of your results. Let the reviewers know that you understand that getting the result you anticipate does not necessarily mean that your hypothesis is right. It just means that the experiment is consistent with your hypothesis; if the result could be consistent with a different hypothesis, can you rule that explanation out with a control. For example, you are testing if protein x is important in fusion of secretory vesicles with the plasma membrane. You are able to inhibit protein x with an available reagent and propose to follow secretion of a tagged marker protein before and after treatment of the cell. If
you see a drop in levels of the secreted marker, you need to outline the controls that rule out non-specific effects of your inhibitor on the cell, or specific effects of your inhibitor on a different step in the secretion pathway.

The overall message for this part of the proposal is that yes, you need to include enough methodological detail to convince the reviewers that you know how the experiment works, but even more importantly you need to show her that you have thought through the possible results of an experiment, can interpret the result of the experiment correctly, and in the case of experimental failure, that you have an alternative approach. Since the space is clearly limited for a short proposal, you may need to develop only one experiment in this kind of detail, showing that you are a careful thinker, and leave other proposed experiments in a sketchier format.

[top]

4. CONCLUSION

You have chosen a topic, found a question that interests you and designed incisive experiments to test some hypothesis you have proposed. All that remains is to summarize your proposed experiment briefly and then point out the wider implications of your work—first in the scope of the field of study, and then, if applicable, in the wider arena. For example:

The proposed studies of PcG mediated repression will not only extend our understanding of the mechanism of transcriptional repression etc etc...., it will also give insight into the general problem of how developmental choices are propagated in a metastable state through multiple cell generations etc etc....

Or,

Studies of the Ty retrotransposon in yeast will give insight into transposon mechanisms etc etc, but may in addition lead to an improved understanding of the cellular processes governing replication of human retroviruses like HIV etc etc...

In other words, this is where you emerge a little from your focused proposal and show that you can think about the broader implications and even applications of your work.

[top]

Summary

The NSF is looking for applications that demonstrate the intellectual merit of the applicant and the broader impact of funding the applicant. Thus, all components of the application receive substantial scrutiny. The reviewers look for intellectual merit principally in your academic record and your proposal. The academic record should demonstrate interest and motivation in studying science. The proposal demonstrates the ability to develop experiments, think logically, and communicate clearly, rather than knowledge of a particular research area. "Broader impacts" refers to the broader impacts of funding an individual who will have a long and productive career, in addition to the broader impacts of the research in the proposal.

Finally, intellectual merit and broader impact are not the only factors in the NSF's decision. There is a geographic component to the NSF fellowships, and if you are a resident in a state other than Maryland it may increase your odds of being selected.

[top]
Remarks of Previous Applicants

Applicant # 1
Honorable Mention, 2005

General Recommendations: Write as clearly and concisely as you can and make your essays as smooth and enjoyable to read as possible. Figure that the reviewers are probably going through hundreds of these and something that is poorly written or confusing might be less favorably received, even if the scientific merit and overall quality of the applicant is high. In contrast, a well-written application might be a breath of fresh air in the middle of the stack and give your application an advantage over the rest.

Get other people to read and provide feedback on all parts of your application. Particularly try to get advice from faculty members and older students who may be familiar with the kinds of questions you address in your proposal and may be able to help you refine your drafts or provide you with advice about what the reviewers might be looking for. Recruit family members or non-scientist friends to read your personal statement and research experience essays to give everything a readability test. Save your drafts and be prepared to accumulate a lot of them. The proposal actually doesn’t seem to be the make-or-break part of your application, so spend time on the other essays as well.

Research Experience: Through your description of your research experience, try to show the reviewers that you have actively sought out research opportunities and that you know what you're getting into with graduate school. Try to convey that you worked on something of significance—not that you necessarily discovered the secret of eternal life in a test tube, but that you had something that was your own project and you weren’t just pouring plates. Also show that you understood the bigger picture of what your projects were about, and that you’re not going to be scared away in graduate school because you didn’t realize that this science thing was often tedious and a lot of work. If you have any publications or you’ve presented at an undergraduate research symposium or anything like that, definitely mention it because that’s also experience they want to know about.

Personal Statement: This is usually my least favorite part of any application, but this is your one opportunity to put a real person behind those pieces of paper. Most applicants will have significant lab experience, be in a good graduate institution, have impressive GPAs and GRE scores, etc., etc., but no one else is you. Show the reviewers your idealism, your sense of humor, your passion—who this young scientist is who aspires to change the world. Have fun writing it—I generally think that if it’s not fun for me to write, it’s not going to be any fun to read, and this is the place where there’s a little more flexibility to have some fun. However, don’t forget your audience—this isn’t a touchy-feely why I want to go to undergrad essay. Everything that you include should have a purpose and should be there to show the reviewer something specific—highlight the aspects of yourself that you think will contribute the most to making you a better scientist. You can be a little creative, but be focused—they seem to like community service, so not everything you include has to be directly related to science. However, as much as possible, play the game and spin all of your qualities so the thought of how good a scientist someone with your attributes is likely to be comes naturally to mind.

Proposal: The hardest thing about writing this proposal will probably be getting it to two pages plus references while still making it thorough and clear. Find the shortest format for listing
references as possible so they don't take up too many lines. Some reviewers will appreciate the space constraints well enough, others will still probably comment on how you failed to address every contingency for experimental failure. Do be sure to address at least the major contingencies for experimental failure, though. Don't implicitly assume that all of the experiments you propose will work as smoothly as butter, because they want to know that you know things often fail or are inconclusive, and that you can think your way around that too. Be prepared to revise a lot and spend a lot of time thinking of the shortest possible way to say things that is still intelligible and gets the point across. Get as many other people to read it as possible and make sure that you fully understand everything that you're proposing to do, or the points that you're unclear about will probably be unclear in the proposal as well. Talking it out with others should help—I usually don't understand anything nearly as well as I think I do or ought to, and other people are likely to point out things you hadn't thought of. It doesn't appear that the proposal is as significant a part of the application as I originally believed, but the NSF still wants to know that you are capable of thinking like a scientist, so you can't blow it off either.

[top]

Applicant # 2
No Award, 2005
Research Experience: I used this section to convince the readers that I knew what I was getting into in graduate school; I heavily emphasized the year I spent at NIH after finishing my undergraduate study.
Personal Statement: This is where I think they look for the "broader impacts" criterion. I failed to convince them of the broader impacts of funding me, mostly because I was trying to make direct connections between their funding and me achieving my goals. I found that difficult, since I intend to achieve my goals regardless of whether or not they fund me now. When I read the reviewers' comments, I realized that the connection didn't have to be direct; I could have attributed every shred of success I might have in the rest of my life to the NSF fellowship. For example, I said that I wanted to do cancer research as a basic scientist, and emphasized the basic science part, lest they not fund me because my interests were too clinical. My reviewers commented that I didn't mention any broader impacts of cancer research but did not indicate that my interests were too clinical. By funding me, they would be funding someone who would contribute to finding cures for cancer in the future – an obvious broader impact which I failed to mention for fear that it was too clinical and too indirectly related to their funding of my graduate research.
Proposal: I think the reviewers look for "intellectual merit" in your academic record, letters of recommendation, and in your proposal. The proposal is when you get to demonstrate that you can think like a scientist. The hard part is that it's impossible to be thorough in two pages; so your objective should be to demonstrate that you can think scientifically rather than to write a proposal that covers every detail in the field and every contingency.

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Resources
Links to NSF Websites
https://www.fastlane.nsf.gov/grfp/

Link to a guide to other predoctoral fellowships
This guide to predoctoral fellowships was prepared entirely by Kip Bitok.
Click here to download the .doc file

Link to online application bank
The research proposal, description of research experience, personal statement, and scoring sheets for each applicant have been bundled into a single PDF file for ease in the downloading process.

NOTICE: Names have been removed from the documents to provide a minimal degree of privacy. The materials contained in this online application bank remain the intellectual property of the individuals who prepared them, and are presented only as examples of previous applications and their degree of success. These materials may only be used as a reference for identifying the criteria by which the NSF evaluates applications, and not for any other purpose. These materials may not be copied or posted in another location without the express permission of the authors.

Applicant 1, Honorable Mention 2005
Applicant 2, No Award 2005
Applicant 3, Honorable Mention 2005
Applicant 4, Honorable Mention 2005

Acknowledgements
Students who have made substantial contributions to this guide include Elizabeth Huang, Seth Zonies, Yuko Oku, Daniel Eyler, and Kip Bitok. Faculty contributors include Brendan Cormack, who generously shared his previously written guide to the research proposal that is included here in its entirety, and Carolyn Machamer. Carol Leibenstein arranged for a server to host this page.
### 2009 GRF Application Schedule and Checklist

#### Suggested Task List for Submitting by the 2009 November Deadline

<table>
<thead>
<tr>
<th>DATE</th>
<th>TASK</th>
</tr>
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<tbody>
<tr>
<td>Sept 1-7</td>
<td>Develop a simple outline for your proposed research plan (question/problem, aims, methods, citations, etc.)</td>
</tr>
<tr>
<td>Sept 8</td>
<td>Set 2-3 dates with your mentor (adviser) to discuss your outline and evolving research essay drafts</td>
</tr>
<tr>
<td>Sept 14-18</td>
<td>Request an official transcript from every higher education institution you have attended (excludes Fall 2009). A hard copy of the official transcript must be sent directly from each institution to the GRFP Operations Center, Suite T-50, 1818 N Street NW, Washington, DC, 20036. Include your full name, GRFP application number, etc.</td>
</tr>
<tr>
<td>Sept 21-25</td>
<td>Contact at least three prospective references to ensure they are available to write letters for you.</td>
</tr>
<tr>
<td>Sept 27</td>
<td>Enter the names of your references into FastLane GRFP system. Send each the required email notification.</td>
</tr>
<tr>
<td>Sept 28</td>
<td>Begin completing the required personal information in the FastLane system for the GRFP.</td>
</tr>
<tr>
<td>Oct 1-2</td>
<td>Create a first draft of your proposed plan of research (essay) from outline. Revise. Review scoring criteria.</td>
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<tr>
<td>Oct 8</td>
<td>If you are uncertain about your data analysis, meet with a statistician to review your research plan.</td>
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<tr>
<td>Oct 9</td>
<td>Submit the draft of your research plan to your mentor for review &amp; feedback. Allow at least 1 week for review.</td>
</tr>
<tr>
<td>Oct 10-15</td>
<td>Create a draft of your personal statement (essay). Allow several days to reflect and rewrite, as this tends to be the hardest essay for students to develop and polish. Ask yourself: Does my writing answer the scoring criteria?</td>
</tr>
<tr>
<td>Oct 16</td>
<td>Give the draft of your non-research essays to family members, friends, study partner, or tutor for feedback.</td>
</tr>
<tr>
<td>Oct 17-18</td>
<td>While waiting for feedback, complete the required information in the GRFP FastLane system.</td>
</tr>
<tr>
<td>Oct 19</td>
<td>Verify that your official transcripts were received by NSF. If not, contact the institution(s) again! Your official transcripts must be received by mail or courier by your discipline's GRFP deadline (below).</td>
</tr>
<tr>
<td>Oct 20-25</td>
<td>Read your essays again. Consider this self scoring rubric (unofficial) to determine if your essays are competitive. Rewrite sections as necessary. Re-check Fastlane sections to assure they are complete. Oct 19-24 is Homecoming Week. Plan on some &quot;down time,&quot; as you may be involved in other activities.</td>
</tr>
<tr>
<td>Oct 26-27</td>
<td>Try to meet with a writing tutor to review your essays (i.e., check grammar, sentence structure, clarity).</td>
</tr>
<tr>
<td>Oct 26-28</td>
<td>Finalize all essays. Check completeness of required information in Fastlane.</td>
</tr>
<tr>
<td>Oct 29-Nov 1</td>
<td>Suggested date range to submit your application. FastLane tends to slow down and &quot;lock up&quot; near deadlines, when too many users are on the system. To avoid possible problems and missing your deadline, submit early.</td>
</tr>
<tr>
<td>Nov 1</td>
<td>Upload your Personal Statement (and one page Extenuating Circumstances essay, if applicable).</td>
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<tr>
<td>Nov 1</td>
<td>Upload Your Previous Research Experience Essay</td>
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<tr>
<td>Nov 1</td>
<td>Upload Your Proposed Plan of Research.</td>
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<tr>
<td>Nov 1</td>
<td>View and print your completed application before submitting. Save the PDF copy.</td>
</tr>
<tr>
<td>Nov 15</td>
<td>Print your confirmation &amp; save! If you do not get a confirmation, call the FastLane Help Desk immediately!</td>
</tr>
<tr>
<td>Nov 30</td>
<td>Absolute deadline for completing GRE tests. (GRE tests are optional, but recommended by NSF and MU)</td>
</tr>
<tr>
<td>Dec 1</td>
<td>Absolute deadline for reference letter writers to submit into the FastLane System.</td>
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</table>

#### Absolute Deadlines for Completed Application and Transcripts to be Submitted in FastLane:

<table>
<thead>
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<th>Date</th>
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</thead>
<tbody>
<tr>
<td>Mon, Nov 2, 2009</td>
<td>Interdisciplinary Fields of Study</td>
</tr>
<tr>
<td>Wed, Nov 4, 2009</td>
<td>Computer &amp; Information</td>
</tr>
<tr>
<td>Wed, Nov 4-6, 2009</td>
<td>Science &amp; Engineering</td>
</tr>
<tr>
<td>Thur, Nov 5, 2009</td>
<td>Geosciences</td>
</tr>
<tr>
<td>Thur, Nov 5, 2009</td>
<td>Psychology</td>
</tr>
<tr>
<td>Thur, Nov 5, 2009</td>
<td>Materials &amp; Manufacturing Sciences</td>
</tr>
<tr>
<td>Thur, Nov 5-6, 2009</td>
<td>Mechanical Engineering</td>
</tr>
<tr>
<td>Fri, Nov 6, 2009</td>
<td>Life Sciences</td>
</tr>
<tr>
<td>Thu, Nov 12, 2009</td>
<td>Engineering</td>
</tr>
</tbody>
</table>

### Notes:

**NOTE:** Allow time for major papers and examinations. If you start later, you will have to adjust these dates accordingly. Follow GRFP guidelines exactly. The NSF does not grant exceptions to their deadlines!

**NOTE:** If you are awarded and accept a 2010 fellowship, notify the fiscal person in your future graduate school immediately. Your institution will need to process internal paper work, in order for you to receive your stipend in the fall semester 2010. NSF does not notify institutions of your award. Your stipend (and the institutions' cost-of-education allowance) will be paid directly to the institution for distribution.
Introduction

The National Science Foundation (NSF)'s Division of Environmental Biology (DEB) and Integrative Organismal Biology (IOB) offer PhD candidates a great opportunity to apply for research money while starting a relationship with NSF. Through its Doctoral Dissertation Improvement Grant (DDIG) program, NSF awards up to $12K to students meeting eligibility requirements. However, these requirements – or any other related logistical matters – are not going to be discussed in this document. We are going to assume that you, the reader, has dutifully researched the logistical components of a successful DDIG (requirements, deadlines, formatting guidelines, various components, etc., available from NSF's webpages www.nsf.gov) and now seek advice on a different level. What separates funded DDIG proposals from the others? How can your DDIG be one of the 20-35% of successful proposals? We have thought about these questions after having served as DDIG panelists – that group of scientists who actually review proposals, rate and debate them, and ultimately recommend the best for funding. After having serving on separate DDIG panels for DEB (Spencer for Ecology, Leonie for Evolution) in Winter 2006, we compared our experiences and noted many common aspects of those proposals that made it to the "fund board" and those that did not. Here is our advice (see several similar points also raised by Skelly 2003):

1. Know your reviewers and your panel
A. Write assuming that your reviewers are tired and not experts in your subdiscipline…. It is important to appreciate that three panelists will read your proposal and many (15-25) others, sometimes at the last minute (even on the airplane!), and often on subjects with which we are familiar but not experts. This situation sets a very high premium on clear, concise writing aimed at a broad ecological or evolutionary audience. Proposals aimed narrowly at a targeted subdiscipline often failed, and nuance often got proposals nowhere.

B. … But be prepared for an expert to review your proposal. Occasionally, a panelist's dissertation or current research overlaps with a student's proposal. In these instances, students who include shallow or murky methodology ran into trouble. So, this means that proposals must be written for a general audience but must also be technically sound.

C. Understand expectations of your panel: Not all NSF programs fund DDIGs. Occasionally, we read proposals arising from other disciplines (e.g., paleontology, genomics) which were more tangentially related to the ecological or evolutionary focus of our panels. If you find yourself in this situation, ensure that you know how your panelists think and tackle questions. Proposals arising from other disciplines failed if they did not pitch research ideas and plans in a way to which reviewers could relate. For instance, ecologists typically expect replication of treatments, statement of hypotheses which can be tested, etc. We both saw genomic-oriented proposals which seemed technically sound and exciting fail in the panels because the proposal did not satisfy these expectations.

2. What are the traits of our favorite proposals? Successful DDIGs:
A. … asked conceptually cutting-edge, often risky questions. The best proposals usually pushed conceptual boundaries and challenged the status quo. This aspect of DDIGs made them very fun to
review. NSF invests relatively few dollars per DDIG ($10K compared to, say, $300K) and therefore is willing to fund exciting proposals which might not work. On the other hand, we saw proposals fail which seemed solid and technically sound but did not excite panelists.

B ... clearly demonstrated the ability to *improve* the grant. Panelists look to see that the dissertation is well in progress, since it is a dissertation improvement grant, not a dissertation grant. In most cases, this means that some compelling data are needed to win over panelists.

C ... broadly pitched the conceptually-motivated introduction on the first page. The reviewer must know what the proposal is about — and want to know more about it — by the end of the first page. Set that hook early; waiting until page three or four is too late.

D. ...tested clearly stated hypotheses which naturally stemmed from the Introduction.

E. ... smoothly integrated background material to place those hypotheses into context. This background material helps to show promise of dissertation.

F. ... very clearly explained methods which obviously related to the hypotheses and strongly argued that the proposed research will answer the questions raised. While panelists are willing to fund risky proposals, they do want to know that the proposed work is logical and feasible.

G. ... contained broader impacts beyond graduate training. The Broader Impacts section offers the student applicant an opportunity to highlight aspects of the research which can appeal to audiences beyond those who will read the student’s papers. You must make an effort to establish the broader implications of your research, whether they are in education and training (especially of underrepresented groups), broader scientific outreach and/or dissemination, establishing scientific partnerships, or societal benefits. Think about ways in which your research can extend to management, education of students of all ages at schools, museums, etc. If you can include such an aspect, do write about it in this section.

H. ... exhibited at least some degree of independence from the advisor’s work and grants. DDIG panelists are not interested in funding the advisor – they want to fund exciting work of promising students. It may be good to strive for some degree of independence from your advisor’s program anyway; it is particularly important for successful DDIGs. This aspect of your DDIG is highlighted in a “Context for Improvement” section. Newly introduced in 2006, it requires that you present a case for how NSF DDIG funding will substantially improve your dissertation while also addressing how your project is distinct and independent from your advisor’s research. We read this section carefully.

3. Some common shortcomings of proposals:
While we read many proposals which truly excited us, we also noticed a common set of problems or mistakes in many others, most of which are completely avoidable. Many of these problems mirror the attributes of our favorite proposals in Section 2. These include:

A. Work that was sound but not terribly exciting. Solid but boring = no funding. Often the problem here is the failure to place research into a broader intellectual and scientific context, or to overemphasize description rather than hypothesis-driven science.

B. Lack of pilot data Preliminary data establishes both that your methodology is sound and appropriate, and that you have the necessary skills to complete the research. The panel must be confident that the research can be done, even if the specific outcome is not yet known. We noticed that proposals with little
or no prior data were rarely funded. If you do not have compelling data yet, consider submitting your proposal in a year.

C. **Overemphasis on Methods, and/or question and inquiry that are not conceptually-rigorous.** Spencer noticed that this problem seemed especially acute with proposals involving newly emerging molecular methods. Yes, these methods are exciting and can open intellectual doors which were formerly closed. However, if your proposal relies heavily on these methods, heed this warning: for ecology and evolution proposals, methods are just means to an end. The end must be feasible, logical research that asks and answers conceptually compelling questions. Spencer saw that poorly framed questions addressed with cutting edge molecular technology were typically denied funding. On the other hand: if your proposal will rely heavily on molecular methods, make sure that you have demonstrated that you can do the work, have developed the necessary primers, have access to PCR machines, etc., in the proposal. Reviewers like conceptually risky proposals but might balk if you seem underprepared to take on an ambitious molecular-technology based program.

D. **Poor scholarship, especially large gaps in knowledge of the literature directly related to the project.** One obvious red flag for this problem is the ‘first time ever’ claim. This claim reads something like, “to our knowledge, this is the first study to examine adaptation in the wild”. Do not make a ‘first time ever’ claim unless you are certain that your study and/or approach is truly unique. Better yet, why not instead emphasize the burning need to answer the question you have raised in your proposal? Panelists are often much more inspired by (and motivated to fund) proposals emphasizing the importance to solve critical problems than those making claims to novelty alone. Many boring questions have not been asked yet...

E. **Poor integration of Introduction, Hypotheses, Background Material, and Methods.** As we said, panelists review up to 25 proposals. They cannot be expected to integrate sections or see the wisdom of certain approaches or questions on their own. The various sections of the proposal must all work together towards a common goal.

F. **Poorly written prose, sloppy presentation.** Do we really need to say it? **Always** do a spell-check before submitting. Also, remember that premium on clear and concise writing....

G. **Minimal or non-existent broader impacts.** This section will generally not kill a scientifically impressive proposal but can sink a proposal on the borderline between Fund and Do Not Fund.

H. **Poorly justified or non-existent ‘context for improvement’.** As with broader impacts, a poor job in this section can kill a borderline proposal. Both of us noticed that when panelists were wavering on a “fund” decision, program officers frequently questioned both this criterion and the broader impacts of the proposal to broker a final decision.

4. **Some more advice:**
   A. **Do not start writing the proposal at the last minute.** It shows and it does not impress.

   B. **Find examples of successful proposals and use them as a model for your own.** This might work especially well if the proposal is similar in style and content to your own. Ask a senior student in your lab group for a copy of her successful DDIG proposal.

   C. **Multiple modes of inference are good if integrated.** Panelists seemed to love work that combined multiple avenues of inference, especially modeling with data collection. We suggest that you highlight this aspect if it applies to your work. However, do not emphasize components that are not well integrated
with the rest of your proposal. An unrelated collection of projects creates the impression that there is no central theme to your research, and that can sink your proposal.

D. Make sure that your advisor reads it. It seemed obvious that many students did not get good feedback from their advisors and/or labmates.

E. Make sure that others read it, especially those who do not study similar problems. If you study plants, give it to a plankton person. If you study disease, give it to someone who works on nutrient cycling. They can note places where the proposal is not clear and does not make sense to the generally informed reader in your overall discipline.

F. Produce high-quality figures. One well thought-out diagram or graph can simultaneously show preliminary data, demonstrate your research skills, and save a substantial amount of text. It can also really help panelists if your proposal involves complex interactions (species, populations, or genes). In this case, look to simplify names of players and include a diagram. You do not want reviewers to get lost with who-is-who.

G. Use the space you have been given. You have eight single-spaced pages for your proposal (excluding the Summary and Context for Improvement sections). Use every bit of it to state your case.

H. Submit a polished document. Go over the proposal one last time before submitting, to correct spelling and or grammatical errors. Even better -- get someone else to read and check the last version of the proposal. This step is particularly important if English is your second language.

I. Note on resubmissions. If your DDIG is not funded the first time around, you should submit it during the following year if possible. (This means that you should prepare to submit a DDIG as soon as you can in your graduate career). The second time around, indicate that the proposal is a resubmission in the text and explain how the proposal incorporated feedback from the previous review. We explicitly looked for that and criticized proposals which ignored previous comments. We knew which proposals were resubmitted, and we accessed prior reviews.

J. Know what NSF wants to know from panelists: As panelists, we are asked to address the following generic questions. Make sure that a reviewer could rate your proposal positively when answering these questions.

What is the intellectual merit of the proposed activity?
How important is the proposed activity to advancing knowledge and understanding within its own field or across different fields? How well qualified is the proposer (individual or team) to conduct the project? (If appropriate, the reviewer will comment on the quality of the prior work.) To what extent does the proposed activity suggest and explore creative and original concepts? How well conceived and organized is the proposed activity? Is there sufficient access to resources?

What are the broader impacts of the proposed activity?
How well does the activity advance discovery and understanding while promoting teaching, training, and learning? How well does the proposed activity broaden the participation of underrepresented groups (e.g., gender, ethnicity, disability, geographic, etc.)? To what extent will it enhance the infrastructure for research and education, such as facilities, instrumentation, networks, and partnerships? Will the results be disseminated broadly to enhance scientific and technological understanding? What may be the benefits of the proposed activity to society?

Literature Cited
<table>
<thead>
<tr>
<th>Program</th>
<th>Program Homepage</th>
<th>Webpage for Doctoral Dissertation Information</th>
<th>Notes</th>
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<tr>
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<tr>
<td>Social Psychology</td>
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Introduction/Overview

I am applying for a postdoctoral fellowship in the Psychophysiology Laboratory in the Department of Psychology at Howard University. I selected Howard University because of its national prominence in producing knowledge about the diversity of the African American experience. Additionally, Dr. Jules Harrell shares my interests in individual differences in responses to psychological challenges and stress and coping in people of African descent. My primary training objectives are: 1) to enhance my theoretical approaches to the study of race-related stress 2) to gain skills in psychophysiology, and 3) to build my evolving program of research. I believe the opportunity to be a fellow at Howard will enhance the innovation and sophistication of my scholarship and prepare me to acquire an assistant professorship and become a lead researcher in the field. Additionally, my research will increase basic knowledge about race-related risk and protective factors, coping, and psychological functioning in African Americans.

Research Objectives, Significance, and Method

Research Objectives

I plan to investigate the relations among racism experiences, racial identity, coping, and psycho-physiological indicators of health (e.g., cardiac reactivity, blood pressure, etc.). The proposed research consists of two phases. The primary aim of the first phase will be to evaluate relations among discrimination experiences, racial identity, coping with discrimination, and changes in psycho-physiological markers of stress/health. The primary research aim of Phase II will be to use qualitative methods to explore how African Americans appraise and cope with experiences of racism. Using qualitative methods will also contribute to the development of empirically validated measures of coping with racism experiences.

The following hypotheses will be tested:

1. Experiences with discrimination will predict changes in cardiac output and blood-pressure, such that individuals who report more frequent discrimination will show more negative changes in cardiac activity.
2. Racial identity will buffer the negative effects of race-related stress experiences on changes in cardiac activity.
3. The relation between discrimination experiences and changes in cardiac activity will be mediated by racial identity (e.g., racial centrality, public regard, etc.), and coping.

Background and Significance

As a group, African Americans are no strangers to racism (Kessler, Mickelson, & Williams, 1999; Landrine & Klonoff, 1996; Thompson, 1996; Williams, Yu, Jackson, & Anderson). Sadly, these experiences with racism continue to remain a significant liability in critical adjustment outcomes such as physical and mental health (Amaro, Russo, & Johnson, 1987; Salgado de Synder, 1987) and overall life satisfaction and well-being (Feagin, 1992; Steele & Aronson, 1995).

While we know that race related factors and coping affect the link between racial discrimination and health, we know little about the mechanisms that account for these
effects. Evidence suggests that race-related person factors such as racial identity (e.g., Neblett, Shelton & Sellers, 2004) and cultural orientation (see Harrell, Hall, & Taliaferro, 2003) moderate the negative impact of racial discrimination on health outcomes. Coping behaviors may also impact the relation between racism and negative health outcomes (Noh & Kaspar, 2003; Harrell, 2000; Scott, 2003, 2004; Williams, Neighbors, & Jackson, 2003). One possibility is that physiological activity mediates the impact of racism on health outcomes. More research is necessary, however, to identify the neural pathways that mediate relations among racist experiences and health (Harrell et al., 2003). The proposed study examines whether the effects of race-related risk and protective factors extend to psycho-physiological indices of health. It also presents an important first step in identifying whether racial identity and individual differences in coping with race-related stress account for changes in psycho-physiological markers of health for African Americans who experience racism.

Given the absence of racial and cultural factors in stress theory and research (Harrell, 2000), and the dearth of empirically validated measures of coping for use with African American populations, qualitative methods may offer several potential benefits. Qualitative methods allow for the discovery of psychological processes that may be inaccessible through traditional quantitative approaches (Charmaz, 1983). These approaches may also facilitate the development of a theoretical framework for coping with racism experiences and help to generate empirically validated measures of coping for use with African American populations.

Method
Study Design and Procedure
The first phase of the study (Year 1) consists of a longitudinal study of racism experiences, race-related protective factors, coping and psycho-physiological indices of health. Participants will complete surveys assessing discrimination experiences, racial identity, and coping and cardiac activity during the first and second semesters of college enrollment. In the second phase of the study (Phase II), I will choose a subset of Phase I study participants (N=20) on the basis of their health (as measured by psycho-physiological indices of health) and racial identity to participate in semi-structured interviews. Phase II participants will reflect low- and high-risk individuals for hypertension as determined by conventional criteria for high blood pressure. Research will be reviewed and approved by the appropriate institutional committees in compliance with Federal policies on research using humans.

Participants
Recruitment of Participants. Participants will be recruited from a list of incoming African American freshman students provided by the university registrar’s office. Students will be contacted by telephone and e-mail during their first month of their first year on campus and asked to participate in a longitudinal study of African Americans’ experiences with racism. During this initial contact, participants will be asked to confirm that they are Black or African American. An explanation of the consent procedures will be given to all participants. Minors will be excluded from the study. African American researchers will administer the research protocol. Participants will be compensated for their participation throughout the study.
Measures

Demographic Questionnaire. This questionnaire will assess demographic characteristics (e.g., age, gender, and socioeconomic status), and covariates of psycho-physiological indices of health (e.g., psychological stress levels).

Multidimensional Inventory of Black Identity (MIBI). The MIBI is a 56-item measure of the three stable dimensions of racial identity (centrality, ideology, and regard) (Sellers, Rowley, Chavous, Shelton, & Smith, 1997). Racial centrality (the extent to which a person defines himself or herself with regard to race) speaks to the significance of race in individuals’ lives. Regard (i.e., affective and evaluative judgments of one’s own race) and ideology (i.e., individual beliefs about how other African Americans should act) reflect the meaning that individuals attribute to race. Participants indicate their agreement with items on a scale from 1 (strongly disagree) to 7 (strongly agree).

Coping. The Agricultural Coping Systems Inventory (ACSI; Utsey, Adams, & Bolden, 2000), is a 30-item measure of culture-specific coping strategies (cognitive/emotional debriefing, collective coping, ritual-centered coping, and ritual-centered coping). Individuals are asked to briefly describe a racist situation that they experienced within the past week or so, and to rate on a 4-point Likert-type scale from 0 = does not apply or did not use to 3 = used a great deal, the extent to which they employed each of the coping behaviors listed.

Perceived Discrimination Scale. Experiences with discrimination will be measured using the Perceived Discrimination Scale (PDS; Harrell, 1997). The PDS consists of 18 items measuring how often the respondent was discriminated against because of their race in the past year (Harrell, 1997). Participants respond on a five-point scale regarding the frequency which they experienced the event (0 = never to 5 = once a week or more).

Psycho-physiological Markers of Health. Resting blood pressure and cardiovascular responses to two psychological stressors will be assessed twice for all study participants.

Qualitative research protocol. In semi-structured interviews lasting 45-60 minutes, participants will be asked to describe their experiences with racism, giving specific examples of two to three personal racist situations. I will also ask participants how much they perceived was at stake, what they felt they could do about it in each situation, and how they coped with the situation. Interviews will be audio-recorded and transcribed.

Analyses

Hierarchical linear regression and structural equation modeling techniques will be used to assess the relations among discrimination experiences, racial identity, coping, and psycho-physiological indicators of health. In Phase II, grounded theory methods (Glaser & Strauss, 1967), which discover theory from data, rather than relying on preconceived hypotheses, will be used to examine patterns and processes through which African American coping responses relate to health status. Confirmatory Factor Analysis will be used to develop a coping scale and assess the factor structure of coping subscales.
Training Objectives and Career Goals

Justification: Sponsoring Institution and Scientist

Howard University has a strong track record in producing African American scholars and scholarship. Additionally, it is committed to recognizing the diversity of the African American experience. Howard’s strong research orientation makes it particularly well-suited for post doctoral training in areas of research that pertain to populations of African descent. The Psychophysiology Laboratory at Howard University represents an additional strength in its selection as a host institution. The laboratory has conducted research using behavioral approaches to health that center upon physiological systems and individual-level personality factors that influence indices of health such as blood pressure in African Americans. This research uses psycho-physiological approaches to examine questions consistent with my current program of research.

In addition to the many benefits that come with selecting Howard University as a sponsoring institution, I am particularly excited about the prospect of working with Dr. Jules Harrell. Dr. Harrell’s current program of research centers upon individual differences in physiological responses to psychological challenges, stress and coping in people of African descent, and behavioral medicine. Dr. Harrell’s conceptualization of cognitive coping styles (Harrell, 1979) was instrumental in the conceptualization of my master’s thesis, which investigated relations among racial identity, perceived stress, coping and adjustment outcomes in African American college freshman. Work by Dr. Harrell examining coping behaviors and styles related to physiological activity (e.g., Clark & Harrell, 1992), protective factors that buffer the effects of racist experiences on health outcomes (Bowen-Reid & Harrell, 2002), and evidence for physiological responses to racism and discrimination (Harrell, et al., 2003) is closely related to my evolving program of research.

Training Objectives

The choice of sponsoring institution and science will help to facilitate the following training goals:

**Goal 1.** My first training objective is to expand my research agenda to incorporate biological determinants of health. While my graduate training has focused primarily on individual/behavioral determinants of health, training as an NSF fellow at Howard would prepare me to examine how multiple contexts (e.g., individual, behavioral, biological and environmental) promote health in African Americans. The proposed research investigates relations among individual factors such as racial identity and stress appraisal, behavioral factors such as coping, biological factors such as blood pressure and cardiovascular reactivity, and social/environmental factors such as racism and discrimination.

**Goal 2.** My second training objective is to augment my skills in psychophysiology. As a Fellow at Howard, I will take courses in psychophysiology and participate in a psycho-physiology research group under the direction of Dr. Harrell. These courses and research opportunities will help to enhance my understanding of how biological factors may impact health in African Americans who experience racism. This training will also enhance the psychological approaches gained during my time as a graduate student at the University of Michigan.
**NSF TRAINING/RESEARCH PLAN**

*Goal 3.* My third training goal is to enhance my methodological and analytic skills. Combining qualitative and quantitative methods will help me to build upon my current skills as a quantitative researcher and help expand my competence with qualitative methods and approaches. I plan to consult with Dr. Cynthia Winston, a personality psychologist at Howard University with considerable expertise in narrative and other qualitative research approaches in working with African American populations.

*Goal 4.* A fourth training goal is to gain exposure to African-centered perspectives/theories that pertain to coping in African Americans. Howard University is an ideal institution for the study of perspectives and approaches to the study of African American populations that may not be taught at predominately White Institutions.

**Career Goals**

My primary career goal is to attain tenure-track professorship at a research-extensive university. As an academic researcher, I intend to examine race-related factors that protect African American health outcomes against the negative effects of race-related stress (e.g., discrimination). I will also study the socio-cultural contexts (e.g., community, neighborhood, and wider social systems) that promote these factors, and the mechanisms by which race-related factors buffer health. I am especially interested in exploring biological mechanisms through which chronic experiences with race-related stress compromise psychological and physical health. As a professor, I will take a leadership role in research and train future generations of students in the concepts and methodology of psychological research, particularly as it relates to racial and ethnic minority populations. As a minority scholar, I expect to advance the field of psychology to include more diverse perspectives in the leadership that build on existing theory to set the national research agenda.

**Career Development and Broad Impact of Fellowship**

The NSF postdoctoral fellowship would help me to augment the participation of underrepresented minorities in U.S. science at the postdoctoral level and to advance my career. As a postdoctoral fellow, I would increase the number of underrepresented minority research scientists. The seminars, courses, and participation in the Psychophysiology Laboratory at Howard University would enhance and broaden my training experiences by exposing me to new concepts, perspectives, and methods that supplement prior training. These experiences would help me to think critically about the connections among multiple determinants of health and explore novel research questions in innovative ways. I believe that critical feedback from Dr. Harrell and other Howard scholars and faculty will prepare me to acquire an assistant professorship and become a lead researcher in the field of ethnic minority health.

Fellowship activities will give me the skills to fill current gaps in knowledge about the interactions among biological, environmental, and social determinants of African American mental health. The proposed research will advance coping research as it examines not only coping behaviors and actions but also biological adaptations to stress. This work is important because the ways in which the body adapts to chronic race-related stress may compromise psycho-physiological systems and perpetuate health disparities in African Americans and other ethnic minority populations. Finally,
fellowship activities will prepare me to produce basic knowledge that will be used to guide intervention and policy efforts that promote minority health.
Example Postdoctoral Researcher Mentoring Plan for an NSF Proposal

[Note: The following mentoring plan is provided as an example; however, the specific mentoring plan a PI develops should fit the project, the school’s goals, and the needs of the postdoctoral researcher(s) to be mentored.]

One postdoctoral researcher will be funded on this project. The postdoctoral researcher’s development will be enhanced through a program of structured mentoring activities. The goal of the mentoring program will be to provide the skills, knowledge and experience to prepare the postdoctoral researcher to excel in his/her career path. To accomplish this goal, the mentoring plan will follow the guidance of the National Academies of Science and Engineering on how to enhance the postdoctoral experience, by providing a structured mentoring plan, career planning assistance, and opportunities to learn a number of career skills such as writing grant proposals, teaching students, writing articles for publication and communication skills [1]. Specific elements of the mentoring plan will include:

- Working with the postdoctoral researcher to establish and implement an Individual Development Plan based on the process developed by the FASEB [2]
- Seminars, workshops and individual consultations on how to identify research funding opportunities and write competitive proposals, offered by the University of California Merced Office of Sponsored Projects
- Participation in seminars and workshops on teaching and learning, as well as access to a teaching mentoring program.
- Opportunities to network with visiting scholars who are leaders in our field by having lunch or dinner with them when they participate in the school’s visiting speaker series
- Participation in a journal club for graduate students and postdocs, in which participants meet weekly, along with a faculty facilitator, to discuss and critique recent journal articles in the field and to discuss how to write and submit journal articles
- Travel to at least two conferences each year [name conferences here] (travel funds are included in the budget), with the goal that the postdoctoral fellow present a poster or paper at the conference.
- Participation in a monthly brown bag lunch series for postdoctoral fellows and graduate students in our school, in which speakers will be invited to discuss subjects related to career development such as how to apply for a faculty position, career paths outside of academia, tips for negotiating salary and start-up funds, how to plan and independent research agenda, etc.
- Participation in the PI’s weekly research group meetings, in which members will be expected to present their research regularly, and feedback and coaching will be given to help all members to develop their communication and presentation skills.

Success of this mentoring plan will be assessed by tracking the progress of the postdoctoral fellow through her/his Individual Development Plan, interviews of the postdoctoral fellow to assess satisfaction with the mentoring program, and tracking of the postdoctoral fellow’s progress toward his/her career goals after finishing the postdoc.


Partner countries

NATO cooperates with a range of countries in different structures. A list of these countries with links to their national information servers.

**Euro-Atlantic Partnership Council (EAPC)**

The EAPC consists of all NATO Member countries and the following partner countries:

- Armenia
- Austria
- Azerbaijan
- Belarus
- Bosnia and Herzegovina
- Finland
- the former Yugoslav Republic of Macedonia
- Georgia
- Ireland
- Kazakhstan
- Kyrgyz Republic
- Malta
- The Republic of Moldova
- Montenegro
- Russia
- Serbia
- Sweden
- Switzerland
- Tajikistan
- Turkmenistan
- Ukraine
- Uzbekistan

**NATO’s Mediterranean Dialogue**

The following seven countries of the Mediterranean region are currently involved:

- Algeria
- Egypt
- Israel
- Jordan
- Mauritania
- Morocco
- Tunisia

**Istanbul Cooperation Initiative (ICI)**

To date, the following four countries of the Gulf Cooperation Council have joined:
**Contact countries**

In addition to its formal partnerships, NATO cooperates with a range of countries which are not part of these structures. Often referred to as "other partners across the globe" or "Contact Countries", they share similar strategic concerns and key Alliance values.

<table>
<thead>
<tr>
<th>Country</th>
<th>Parliament</th>
<th>GOV</th>
<th>State Head</th>
<th>PM</th>
<th>MFA</th>
<th>Info Centre</th>
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<tr>
<td>Australia</td>
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<td>Japan</td>
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<td>Republic of Korea</td>
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<tr>
<td>New Zealand</td>
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**Legend**

- **1St**: Parliament, 1st Chamber
- **Sen**: Senate, 2nd Chamber
- **GOV**: Government
- **State Head**: Head of State / President
- **PM**: Prime Minister
- **MFA**: Ministry of Foreign Affairs
- **Ministry/Department of Defence**
- **Military / Chief of Staff**
- **National Mission or Delegation to NATO**
- **Info Centre**

1. Turkey recognises the Republic of Macedonia with its constitutional name.
Goals, Hypotheses, Research Questions, and Project Objectives

A. Three ways to begin an academic research project:

1. A goal statement or statements
2. An hypothesis or hypotheses
3. Research questions

1. Goal: A general statement of a project’s ultimate purpose. It can be open-ended, idealistic, even visionary.
   - Our goal with this project is to improve the teaching of STEM subjects (science, technology, engineering, mathematics) to undergraduates.
   - This project will deepen our understanding of the effects of global climate change on populations of saltwater plankton.
   - This study will result in the first complete database for the assessment of the effects of toxic metals on human reproduction.
   - We seek to understand how cell division and differentiation are regulated by a myriad of extracellular and intracellular signals.
   - The research team will develop an analytical framework for classifying brain potential analysis of motor functions and decision making.

2. Hypothesis: A specific, testable assumption or conjecture that the research data are expected to support. (Note: Whether the data do or do not support the hypothesis is of equal scientific importance!)
   - We will test the hypothesis that large neurons are selectively destroyed in Alzheimer's Disease by measuring the sizes of ganglion cells in retinas of AD patients compared with those in age-matched control groups.
   - Magnesium deficiency caused by glucose intolerance, insulin resistance, or other factors in hypertensive patients leads to increased vasomotor tone via altered release of vasoactive cyclooxygenase and lipoxygenase products.
   - For infection to occur, the capsids of parvoviruses undergo structural variation through the binding or release of divalent ions, by site-specific proteolysis, or by variation in specific intra- or inter-chain bonds.
   - We hypothesize that moderate consumption of fruits grown in Oman over the routine baseline intake would reduce the progression of cognitive impairment inpatients diagnosed with Alzheimer's Disease (AD) and mild cognitive impairment (MCI).
3. Research Questions: Inquiries related to lack of knowledge in an important area of research, answers to which are sought through the research design.

- Are there common lifestyle traits among those suffering from diabetes in this region?
- Do local physicians address these traits in prescribing treatment and follow up regimens?
- Are there significant variations in standards of practice among physicians?
- If so, is there a rational basis for these variations?

B. Project objective: A specific, measurable, benchmark, milepost, or outcome achieved in moving toward the goal.

- Whether starting with a goal, an hypothesis, or research questions, the next step is to formulate the project's objectives
- Most successful research designs are driven by 2 – 4 objectives
- Active verbs are key to writing strong objectives
- Upon completion of the final objective, the goal should be obtained (or at least major progress should be achieved)
- In health-related research, objectives are often referred to as “Specific Aims” (US National Institutes of Health)
- Examples:
  - We will design and test ten new modules for teaching cardiopulmonary resuscitation to first year nursing students
  - Utilize a modeling approach to characterize pharmacokinetic data and factors influencing tacrolimus dosage among the target population
  - Determine the potential involvement of the calcium-sensitive process in the development of myogenic tone in both young and aged coronary arteries
  - To identify the primary means parents use to discipline their children, and rank them by level of violence
Pre & Postdoctoral Career Development: NIH Fellowships and Grants

Robert Porter, PhD
Grant-Winners Seminars
Knoxville TN
reporter@grant-winners.com

Goal:

To provide guidance on applications for NIH postdoctoral career awards:
- Fellowships ("F") awards
- Career transition awards ("K")
- K99/R00 "Pathway to Independence"
Workshop Objectives

To help you:

- Choose the appropriate award track for your career stage and future goals
- Understand the application requirements for a NIH fellowship or career transition award
- Identify effective strategies for developing a successful fellowship or grant proposal

NIH Grant/Career Timeline

Training:
- F30
- F31
- F32
- K Awards (career dev)

Research:
- R01, R03, R21
- P01
NIH Fellowship (F) Awards

Provide support for pre- and postdoctoral studies in disciplines supported by NIH:

- Tuition: percentage varies by program
- Stipend: varies by years of experience
- Health insurance: individual and family
- Institutional allowance: research-related expenses
- Eligibility: US citizens or Permanent Residents

Note: Not all Institutes support F tracks; check Program announcements!

NIH Fellowship (F) Awards

F Award Tracks:

- F30: predoctoral award for combined M.D./Ph.D. training
- F31: predoctoral award to promote diversity in health-related research (Ph.D., M.D./Ph.D., BSN/Ph.D, and other combinations)
- F32: promising postdoctoral applicants with high potential to become independent investigators

See “F Kiosk” page:
http://grants.nih.gov/training/F_files_nrsa.htm
K Awards:

- Support for career transition (~ 2% NIH budget)
- Wide range of types: currently 14 (K01-K99)
- For clinicians & basic scientists
- For junior & senior faculty
- Foster basic, clinical & patient-oriented research
- Provide partial funding for salaries
- Application success rates: 35 – 40%

K Awards

1. For **mentored** career development:
   - Basic scientists: K01
   - Clinicians: K08, K23, K24

2. For career **transition**:
   - Basic scientists: K02, K22
   - Pathway to Independence: K99/R00
K Awards for basic scientists

<table>
<thead>
<tr>
<th>Ph.D.</th>
<th>Postdoc.</th>
<th>Faculty —&gt; Independent investigator</th>
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</table>

K01 Mentored Research Scientist Award

K02 Independent Scientist Award

K22 Career Transition Award

K Awards

Mentored career development:

- Development of junior faculty
- Dedicated mentor is essential for
  - Successful application
  - Successful outcome
- Basic scientists & clinicians
K Awards for basic scientists

K01: Mentored Research Scientist Award

"Career development in a new area of research..."

- Potential for productive independent research
- Mentor with extensive research experience
- 75% effort over 3-5 years
- Differences among Institutes

K Awards for basic scientists

K02: Independent Scientist Award

"Develop career of funded scientists..."

- Salary support for newly independent scientists
- Must have peer-reviewed research support
- 75% effort for 5 years
K Awards for basic scientists

K22: Career Transition Award

"Support for postdoctoral fellows in transition to faculty positions"

- Potential for productive independent research
- Differences among Institutes: may involve training in intramural NIH programs

K99/R00 (Kangaroo) Award

- NIH Pathway to Independence (PI) Program
- **Goal:** To accelerate transition from a postdoctoral status to an independent scientist capable of receiving an R01 award
- Both clinicians and basic scientists are eligible
- Provides up to five years of support consisting of two phases:
  1. Initial 1-2 years of mentored support for highly promising postdoctoral research scientists (K99 Phase)
  2. Followed by up to 3 years of independent support contingent on securing an independent research position (R00 Phase)
Mentored (K99) Phase

- Provides 1-2 years mentored support for highly promising postdoctoral research scientists with terminal clinical or research doctorates
- Total cost per year up to $90,000
- Only US institutions may apply on behalf of candidates

U.S. citizens and non-U.S. citizens eligible

Independent Investigator (R00) Phase

- Transition from K99 to R00 (years 3-5) is to be continuous in time
- Start of R00 Phase requires a tenure-track, full time assistant professor position (or equivalent)
- Transition is subject to NIH review and evaluation of research plan
- Total support up to $250,000 per year
- Institution must demonstrate commitment to candidate (minimum 75% effort, space, equipment, etc.)
- PI expected to apply for independent grant support
## Comparison of K22 and K99/R00 Awards

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<thead>
<tr>
<th></th>
<th>K22</th>
<th>K99/R00</th>
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<tbody>
<tr>
<td>Transition Award (postdoc to faculty)?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Duration?</td>
<td>Varies by IC</td>
<td>NIH: 5 yrs</td>
</tr>
<tr>
<td>U.S. Citizenship/ Green card?</td>
<td>Required</td>
<td>Not Required</td>
</tr>
<tr>
<td>Mentored Phase</td>
<td>Varies by IC</td>
<td>2 yr</td>
</tr>
<tr>
<td>Independent Phase</td>
<td>Varies by IC</td>
<td>3 yrs</td>
</tr>
<tr>
<td>Awardee can go to foreign institution?</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cost</td>
<td>Varies by IC</td>
<td>$90K yrs 1-2 (TC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$747K yrs 3-5 (TC)</td>
</tr>
<tr>
<td>F &amp; A Costs</td>
<td>8%</td>
<td>Up to 50%</td>
</tr>
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</table>

## Selecting Your K Award Track

- **“K kiosk”**
  
  [http://grants1.nih.gov/training/careerdevelopmentawards.htm](http://grants1.nih.gov/training/careerdevelopmentawards.htm)
  Thumbnail sketches of all “K” tracks with links to full announcements

- **“Career Award Wizard”**
  Answer questions in sequence to arrive at most appropriate track(s)
NIH Career Transition Awards

Anatomy of an Application

Warning: Electronic Submission required!

http://grants.nih.gov/grants/funding/submissionschedule.htm
Project Description (Abstract)

- PURPOSE: Describe succinctly every major aspect of proposed project except budget
- Length: 1/2 page (space provided)
- Should touch briefly on:
  - Background and significance of proposed research
  - Specific aims or hypothesis
  - Unique features of project
  - Methodology (action steps) to be used
  - Expected results
  - Evaluation methods

The most important part of your application! Must sell the reviewers on you, your career plan, and your proposed research
1. The Candidate:

- **Candidate's Background**
  - Additional information not in biosketch

- **Career Goals & Objectives**
  - "Scientific Biography"
  - How training will fit career development

- **Career Development/Training Activities**
  - New skills & knowledge to be learned
  - Must include training in:

  Responsible Conduct in Research (RCR)

---

2. Statement(s) by Mentor and Sponsor(s):

- **Description of Training Program**
  - Include activities other than research
  - Sponsor's experience as mentor
  - Concurrent responsibilities
  - Assurance of release from duties
  - Source of support for research project

- **List other collaborators, consultants**
  - Provide letters from each
3. Environment & Institutional Commitment:

• **Description of Institutional Environment**
  - Strong relevant research program
  - Availability of resources
  - Intellectual interactions

• **Institutional Commitment**
  - Adequate support from institution
  - Adequate resources (lab, office, etc.)
  - Commitment to candidate
  - Agreement must be signed by appropriate Institutional Official

4. Research Plan:

• Statement of Hypothesis & Specific Aims
• Background, Significance & Rationale
• Preliminary Studies & Any Results
• Research Design & Methods
Crafting a Successful Proposal

Provide clear, concise answers to key questions:

- Why is this study important?
- Are the experiments feasible?
- What will be accomplished?
- How will it change the field?

Crafting a Successful Proposal

Design a clear experimental plan:

- Devise a clearly stated, testable hypothesis, followed by 2 – 4 specific aims (research objectives)
- Keep rest of proposal focused on this structure
- Describe outcomes: What will you learn?
- Anticipate pitfalls: outline alternatives
- Provide a timeline: Limit experiments to what can be accomplished within the time period
K Award Application

Reference Letters:
- Required for K01, K08, K22, K23 and K99 (mentored) applications
- Three (3) letters from individuals other than those involved in the application, i.e., not sponsor/mentor or collaborators
- Should address candidate's competence & potential as an independent investigator
- Use NIH form letter to request letters of reference

Tips for Best Reference Letters

- Develop effective working relationships with potential referees
- Keep your referees updated on your progress
- Make your referees' job easy, provide:
  - Current CV, reprints of papers
  - Draft of proposal

Remember: This is a personal and professional relationship that may last your entire career!
K Awards: Review Criteria

1. Candidate
2. Career development plan
3. Research plan
4. Mentor
5. Environment & Institutional commitment
6. Budget

How will your proposal be reviewed?

New videos:

NIH Tips for Applicants
Discover how to make your application more competitive.

www.youtube.com/watch?v=9chRMsCGIf0

NIH Peer Review Revealed
A front-row seat to a peer review meeting.

www.youtube.com/watch?v=fBDx6I6dQA
"The meek may inherit the earth, but not the grant dollars."
- J. Paul Getty
Specific Aims (Tests of the Hypotheses)

Hypotheses

Having established the problem and a logical sequence within which it can be considered, one now considers specific hypotheses that should be tested.

The Abstract and Specific Aims
The Abstract and Specific Aims

The purpose of the proposed research is to determine whether or not a clear relationship exists between the title for the Special Aims. The first line is excellent but the rest of the title would have been more descriptive if the specific details were included.

Specific Aim 1 is to compare the prevalence and window dressing. What is the appropriate way to describe the prevalence? Does the proposed research address this aim? Could the terms be defined more clearly?

Specific Aim 2 is to compare the prevalence and window dressing. What is the appropriate way to describe the prevalence? Does the proposed research address this aim? Could the terms be defined more clearly?

The proposed research is that without a clear relationship to the proposal, the data will be generated. Significant correlations between the effect of age on the visual field. The specific aims are to determine the extent through preclinical intervention and statistical associations of the proposed research. We hypothesize that the proposed research will be conducted with appropriate controls. The specific aims are to determine the extent through preclinical intervention and statistical associations of the proposed research. We hypothesize that the proposed research will be conducted with appropriate controls.

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THE ABSTRACT

The following is a relatively well-written Specific Aim:

IRR (Interleukin-1R) antagonist

- The following paragraph describes the specific aims of the study.

1. To determine how cytokine networks influence human RFP neurotransmission.

2. To characterize cytokine networks in human RFP neurotransmission.

3. To determine how cytokine networks influence human RFP neurotransmission.

In this study, we propose to address the following specific aims:

1. To determine how cytokine networks influence human RFP neurotransmission.

2. To characterize cytokine networks in human RFP neurotransmission.

3. To determine how cytokine networks influence human RFP neurotransmission.

The following section describes the specific aims of the study.

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THE ABSTRACT AND SPECIFIC AIMS

(see below)"...

Significance: These studies will provide insight into mechanisms with hypertension or hypotension/hypertension.

Specific Aims:

1. Determine the effects of low Mg on vascular and extracellular functions and how these changes may play an important role in the development of hypertension/hypertension.


3. Determine the effect of Mg level on Mg-specific function and insulin resistance.

4. Determine the effect of Mg level on Mg-specific function and insulin resistance.

5. Determine the effect of Mg level on Mg-specific function and insulin resistance.

6. Determine the effect of Mg level on Mg-specific function and insulin resistance.

The proposed work is also presented, but if the appearance test.
NIH F31/F32 FELLOWSHIP APPLICATION DEVELOPMENT

1. **Identify** federal institute or private foundation with stated missions confluent with proposed research;
2. **Review** funding announcement and notify Research Office of intent to submit;
3. **Meet** with advisor/sponsor, Director of Statistics Core, Grant Analyst

**NRSA APPLICATION COMPONENTS**

I. **Research Training Plan Components**

   **Cover Letter: No more than one page.**
   Must include 1) Application title; 2) Funding Opportunity title and number; 3) Statement that you have attached any required agency approval documentation for the type of application submitted; 4) list of Referees including the names, degrees, and affiliations of the individuals from whom you have asked to submit reference letters. Optionally, include: 1) disciplines involved, if multidisciplinary; 2) Request of an assignment (referral) to a particular Institute/Center or Scientific Review Group; 3) list of individuals (e.g., competitors) who should not review your application and why.

   **Project Summary/Abstract: No more than 30 lines of text.**
   The Abstract must contain a summary of the proposed activity suitable for dissemination to the public. It should be a self-contained description of the project and should contain a statement of objectives and methods to be employed. It should be informative to other persons working in the same or related fields and insofar as possible understandable to a scientifically or technically literate lay reader. The Project Summary is meant to serve as a succinct and accurate description of the proposed work when separated from the application. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project (i.e., relevance to the mission of the agency). Describe concisely the research training program design and methods for achieving the stated goals. Avoid describing past accomplishments and the use of the first person. Finally, please make every effort to be succinct. Do not include proprietary, confidential information or trade secrets in the description section, since this will become public information if the project is funded.

   **Project Narrative: No more than three sentences.**
   Describe the relevance of this research to public health. In this section, be succinct and use plain language that can be understood by a general, lay audience.

   **Specific Aims: Specific Aims are limited to one page.**
   State concisely the goals of the proposed research and summarize the expected outcome(s), including the impact that the results of the proposed research will exert on the research field(s) involved. List succinctly the specific objectives of the research proposed, e.g., to test a stated hypothesis, create a novel design, solve a specific problem, challenge an existing paradigm or clinical practice, address a critical barrier to progress in the field, or develop new technology.
**Research Strategy: Research Strategy is limited to six pages.**
The Research Training Plan should include sufficient information needed for evaluation of the project, independent of any other document. Be specific and informative, and avoid redundancies. This section should be well-formulated and presented in sufficient detail that it can be evaluated for both its research training potential and scientific merit. It is important that it be developed in collaboration with your sponsor, but it should be written by you, the fellowship applicant. All figures and photographs should be included inline in the strategy, not in the Appendix or elsewhere in the application. Be succinct and remember that there is no requirement to use all six pages allotted. Internet Web site addresses (URLs) may not be used to provide information necessary to the review, except for reference citations, because reviewers are under no obligation to view the Internet sites. Organize the Research Strategy in the specified order using the instructions provided below. Start each section with the appropriate section heading — Significance, Approach and Preliminary Studies (if applicable). Cite published experimental details in the Research Strategy section and provide the full reference in the Bibliography and References Cited.

**Significance**
- Explain the importance of the problem or critical barrier to progress in the field that the proposed project addresses.
- Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
- Describe how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.

**Approach**
- Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims of the project. Include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate.
- Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the aims.
- If the project is in the early stages of development, describe any strategy to establish feasibility, and address the management of any high risk aspects of the proposed work.
- Point out any procedures, situations, or materials that may be hazardous to personnel and precautions to be exercised. A full discussion on the use of select agents should appear in a separate section.
- Include any courses that you plan to take to support the research training experience.

If an applicant has multiple Specific Aims, then the applicant may address Significance and Approach for each Specific Aim individually or collectively.

**Preliminary Studies:** include information on preliminary studies, if any. Discuss the applicant's preliminary studies, data and/or experience pertinent to this application. When applicable, provide a succinct account of published and unpublished results, indicating progress toward their achievement.

**Appendix:** no page limit, but do not use the Appendix to circumvent the page limitations of any section for which a page limit applies.
Do not include photographs or color images of gels, micrographs, etc. The Appendix can include up to three publications if accepted/published and not publicly available. A summary sheet is encouraged. Patents, surveys, questionnaires, and other data collection instruments; clinical protocols, and informed consent documents may be submitted in the Appendix as necessary.

**References:** no page limit.
Must include PMCID numbers where available. If you use Endnote, import citations from PubMed, download the NIH output style from the Endnote website, and this will be done automatically.

**Special disclosures/sections required if you are conducting:** Clinical Trials; Phase-III Clinical Trials; vertebrate animals research; select agent research; research on model organisms; genome-wide assay studies; stem cell research. See full instruction set if your research includes these elements.
II. Applicant, Institution and Sponsor Information

Sponsor and Co-Sponsor Information: *This item is limited to six pages.* The sponsor should be an active investigator in the area of the proposed research training and be committed both to the research training of the Fellowship Applicant and to the direct supervision the applicant's research. The sponsor must document the availability of sufficient research support and facilities for high-quality research training. The sponsor, or a member of the mentoring team, should have a successful track record of mentoring predoctoral students. Applicants are encouraged to identify more than one mentor, i.e., a mentoring team, if this is deemed advantageous for providing expert advice in all aspects of the research and training program. In such cases, one individual must be identified as the principal sponsor who will coordinate the applicant’s research training program. The applicant must work with his/her sponsor(s) in preparing the application. The sponsor should describe the research training plan for the applicant (coordinated with the applicant’s research strategy). The sponsor and any co-sponsors are also expected to provide an assessment of the applicant’s qualifications and potential for a research career. The research environment and the availability and quality of needed research facilities and research resources (e.g., equipment, laboratory space, computer time, available research support, etc.) must also be described. The description should include items such as classes, seminars, and opportunities for interaction with other groups and scientists. Training in career skills, e.g. grant-writing and presentation skills are strongly encouraged.

1. **Research Support Available:** In a table, list all current and pending research and research training support specifically available to the applicant for this particular training experience. Include funding source, complete identifying number, title of the research or training program, and name of the principal investigator, dates, and amount of the award. Include this information for any co-sponsor as well.

2. **Sponsor's/Co-Sponsor's Previous Fellows/Trainees:** Give the total number of predoctoral and postdoctoral individuals previously sponsored. Select up to five that are representative and, for those five, provide their present employing organizations and position titles or occupations. Include this information for any co-sponsor as well.

3. **Training Plan, Environment, Research Facilities:** Describe the research training plan that you have developed specifically for the Fellowship applicant. Include items such as classes, seminars, and opportunities for interaction with other groups and scientists. Describe the research environment and available research facilities and equipment. Indicate the relationship of the proposed research training to the applicant's career goals. Describe the skills and techniques that the applicant will learn. Relate these to the applicant's career goals. The description should include items such as classes, seminars, and opportunities for interaction with other groups and scientists. Training in career skills, e.g. grant-writing and presentation skills are strongly encouraged.

4. **Number of Fellows/Trainees to be Supervised During the Fellowship:** Indicate whether pre- or postdoctoral. Include this information for any co-sponsor as well.

5. **Applicant's Qualifications and Potential for a Research Career:** Describe how the Fellowship applicant is suited for this research training opportunity based on his/her academic record and research experience level, including how the research training plan, and your own expertise as the sponsor will assist in producing an independent researcher.

**Selection of Sponsor and Institution:** *This item is limited to one page.*

1. **Explain why the sponsor, co-sponsor (if any), and institution were selected to accomplish the research training goals.** If the proposed research training is to take place at a site other than the sponsoring organization, provide an explanation here.

2. **Foreign Institution.** If you are proposing a research training experience at a foreign institution, show that the foreign institution and sponsor offer special opportunities for training that are not currently available in the United States. Key factors in the selection of a foreign institution should be described. If applicable, the need for and level of proficiency in reading, speaking, and comprehending the foreign language should be addressed.

**Respective Contributions:** *This item is limited to one page.*

Describe the collaborative process between you and your sponsor/co-sponsor in the development, review, and editing of this research training plan. Discuss the respective roles in accomplishing the proposed research.

**Biographical Sketches for Applicant, Sponsor, and co-Sponsors:** *limited to four pages each.*

Each sketch is comprised of 1) Personal Statement, 2) Positions and Honors, 3) 15 peer-reviewed publications most relevant to the proposal, and 4) Research Support. See PHS 398 sample and template.
Fellowship References (3-5): The sponsor cannot be counted as a reference. Electronic submission of a Fellowship Reference Form is a separate process from submitting an application electronically. Fellowship Reference Forms are due by the application receipt deadline date. Fellowship Reference Forms are submitted directly through the eRA Commons, while the application is submitted through Grants.gov. Applicants must arrange to have at least three (and no more than five) references submitting using the Fellowship Reference Form on their behalf to the eRA Commons Web site. All fellowship applicants must include a list of Referees in the Cover Letter. The list must include the names, degrees, and affiliations of the individuals from whom you have asked to submit reference letters. At least three references are required. Your references should be carefully selected. Only those individuals who can make the most meaningful comments about your qualifications for a research career should be used. Whenever possible, select at least one referee who is not in your current department. If not submitting a reference from the dissertation advisor or chief of service, provide an explanation. Request reference reports only from individuals who will be able to submit them in time. Consider any factor (e.g., illness or extended vacation) that might cause an inordinate delay. Give these reference forms to the referees well in advance of the application due date. Technical submission instructions for referees can be found in the Individual Fellowship Application Guide SF424 (R&R), Section 5.4, Part B. The referees must provide information including (a) the PD/PI (Fellowship applicant) Commons user name, (b) the PD/PI first and last name as they appear on the PD/PI’s Commons account, and (c) the Funding Opportunity Announcement number in order for the references to be matched to the application.

Responsible Conduct of Research: This item is limited to one page.
Every fellow must receive instruction in the responsible conduct of research appropriate to the career stage of the applicant. The section must document prior participation or instruction in responsible conduct of research during the applicant's current career stage (including the date instruction was last completed) and propose a plan to either receive instruction in responsible conduct of research or participate as a course lecturer, etc., depending on the applicant's career stage. Applications must include a description, limited to no more than one page, of the sponsoring institution’s plans to provide, and the fellowship applicant’s plans for obtaining, instruction in the responsible conduct of research, including the format, subject matter, faculty participation, duration and frequency of instruction. The plan should be tailored to the needs of the fellow, and may go beyond formal institutional courses and provide opportunities for the individual to develop their own scholarly understanding of the ethical issues associated with their research activities and their impact on society. The role of the sponsor in the instruction in responsible conduct of research must be described.

Goals for Fellowship Training and Career: This item is limited to one page.
The fellowship applicant must describe his/her overall career goals, and explain how the proposed research training will enable the attainment of these goals. Identify the skills, theories, conceptual approaches, etc. to be learned or enhanced during the award.

Activities Planned Under This Award: This item is limited to one page.
The fellowship applicant must describe by year the activities (research, coursework, etc.) s/he will be involved in under the proposed award and estimate the percentage of time to be devoted to each activity, based on a normal working day for a full-time fellow as defined by the sponsoring institution. The percentage should total 100 for each year. Also, briefly explain activities other than research and relate them to the proposed research training. Predoctoral fellowships (F31) may reflect up to five years.

Doctoral Dissertation and Research Experience: This item is limited to two pages.
Summarize your research experience (limited to 2 pages) in chronological order. Advanced graduate students, who have (or will have) completed their comprehensive examinations by the time of award must also include a narrative of their doctoral dissertation (may be preliminary). If you have no research experience, list other scientific experience. Do not list academic courses. In summarizing their research experience, Postdoctoral and Senior Fellowship applicants should include the areas studied and conclusions drawn. Postdoctoral fellowship applicants should also specify which areas of research were part of their thesis or dissertation and which, if any, were part of a previous postdoctoral project.
III. Human Subjects Research

For all research involving human subjects, a part of the peer review process will include careful consideration of protections from research risks, as well as the appropriate inclusion of women, minorities, and children. The Scientific Review Group (SRG) will assess the adequacy of safeguards of the rights and welfare of research participants, and the appropriate inclusion of women, minorities, and children, based on the information in the application. The evaluation of the inclusion plans will be factored into the overall score that the SRGs award for scientific and technical merit of the application. Much of the information on the protection of human subjects that you are required to provide in the Fellowship application is identical to information that you will be required to provide for IRB review at your own institution.

Protection of Human Subjects: no page limitation. This component consists of the four following sections.

1. Risks to Human Subjects
   a. Human Subjects Involvement and Characteristics, and Design
      • Describe the proposed involvement of human subjects in the work outlined in the Research Strategy section.
      • Describe and justify the characteristics of the subject population, including their anticipated number, age range, and health status if relevant.
      • Describe and justify the sampling plan, as well as the recruitment and retention strategies and the criteria for inclusion or exclusion of any subpopulation.
      • Explain the rationale for the involvement of special vulnerable populations, such as fetuses, neonates, pregnant women, children, prisoners (including those in detention or otherwise institutionalized, and those who are incarcerated during the course of the study), or others who may be considered vulnerable.
      • If relevant to the proposed research, describe procedures for assignment to a study group. As related to human subjects protection, describe and justify the selection of an intervention’s dose, frequency, and administration.
      • List any collaborating sites where human subjects research will be performed, and describe the role of those sites and collaborating investigators in performing the proposed research. Explain how data from the site(s) will be obtained, managed, and protected.
   b. Sources of Materials
      • Describe the research material obtained from living individuals in the form of specimens, records, or data.
      • Describe any data that will be collected from human subjects.
      • Indicate who will have access to individually identifiable private information about human subjects.
      • Provide information about how the specimens, records, and/or data are collected, managed, and protected as well as whether material or data that include individually identifiable private information will be collected specifically for the proposed research project.
   c. Potential Risks
      • Describe the potential risks to subjects (physical, psychological, financial, legal, or other), and assess their likelihood and seriousness to the human subjects.
      • Where appropriate, describe alternative treatments and procedures, including the risks and potential benefits of the alternative treatments and procedures, to participants in the proposed research.

2. Adequacy of Protection Against Risks
   a. Recruitment and Informed Consent
      • Describe plans for the recruitment of subjects (where appropriate) and the process for obtaining informed consent. If the proposed studies will include children, describe the process for meeting requirements for parental permission and child assent.
      • Include a description of the circumstances under which consent will be sought and obtained, who will seek it, the nature of the information to be provided to prospective subjects, and the method of documenting consent. If a waiver of some or all of the elements of informed consent will be sought, provide justification for the waiver. Informed consent document(s) need not be submitted to the PHS agencies unless requested.
   b. Protections Against Risk
      • Describe planned procedures for protecting against or minimizing potential risks, including risks to privacy of individuals or confidentiality of data, and assess their likely effectiveness.
      • Research involving vulnerable populations, as described in the DHHS regulations, Subparts B-D must include additional protections. Refer to additional instructions for Pregnant Women, Human Fetuses and Neonates; Prisoners; and Children.
• Where appropriate, discuss plans for ensuring necessary medical or professional intervention in the event of adverse effects to the subjects. Studies that involve clinical trials (biomedical and behavioral intervention studies) must include a general description of the plan for data and safety monitoring of clinical trials and adverse event reporting to the IRB, the NIH and others, as appropriate, to ensure the safety of subjects.

3. Potential Benefits of the Proposed Research to Human Subjects and Others
   • Discuss the potential benefits of the research to research participants and others.
   • Discuss why the risks to subjects are reasonable in relation to the benefits to research participants and others.

4. Importance of the Knowledge to be Gained
   • Discuss the importance of the knowledge gained or to be gained as a result of the proposed research.
   • Discuss why the risks to subjects are reasonable in relation to the importance of the knowledge that reasonably may be expected to result.

Targeted/Planned Enrollment Table: All new clinical research studies should collect and report information on participants with respect to two categories of ethnicity and five categories of race. Investigators should report: (a) the number of research participants in each ethnic category; (b) the number of research participants who selected only one category for each of the five racial categories; (c) the total number of research participants who selected multiple racial categories reported as the “number selecting more than one race,” and (d) the number of research participants in each racial category who are Hispanic or Latino. Investigators may provide the detailed distributions, including all possible combinations, of multiple responses to the racial designations as additional information. The full instructions address ways to document use of existing data and data collected at foreign sites.

Inclusion of Women and Minorities: no page limitation, but be succinct. Scientific Review Groups will assess each application as being acceptable or unacceptable with regard to the inclusion of women and minorities in clinical research. Address, at a minimum, the following four points:
1. The targeted/planned distribution of subjects by sex/gender and racial/ethnic groups for each proposed study or protocol using the format in the Targeted/Planned Enrollment Table. (Instructions for completing this table are provided below in 4.3.) If using existing specimens and/or data without access to information on the distribution of women and minorities, so state and explain the impact on the goals of the research as part of the rationale that inclusion cannot be described (item 3 below). Alternatively, describe the gender and minority composition of the population base from whom the specimens and/or data will be obtained. Include the Targeted/Planned Enrollment Tables in this section.
2. A description of the subject selection criteria and rationale for selection of sex/gender and racial/ethnic group members in terms of the scientific objectives and proposed study design. The description may include, but is not limited to, information on the population characteristics of the disease or condition under study.
3. A compelling rationale for proposed exclusion of any sex/gender or racial/ethnic group (see the full application instructions for examples pertaining to various exclusion scenarios).
4. A description of proposed outreach programs for recruiting sex/gender and racial/ethnic group members as subjects.

Inclusion of Children: no page limitation, but be succinct.
• Provide either a description of the plans to include children, or, if children will be excluded from the proposed research, application, or proposal, present an acceptable justification for the exclusion (see the full instructions for examples pertaining to various exclusion scenarios).
• If children are included, the description of the plan should include a rationale for selecting a specific age range of children. The plan also must include a description of the expertise of the investigative team for working with children at the ages included, of the appropriateness of the available facilities to accommodate the children, and the inclusion of a sufficient number of children to contribute to a meaningful analysis relative to the purpose of the study.

For the purposes of this policy, all individuals under 21 are considered children; however, exclusion of any specific age group, such as individuals under 18, should be justified in this section. It is expected that children will be included in all clinical research unless one or more of the following exclusionary circumstances apply: 1) the research topic to be studied is not relevant to children; 2) Laws or regulations bar the inclusion of children in the research; 3) the knowledge being sought in the research is already available for children or will be obtained from another ongoing study, and an additional study will be needlessly redundant. See the full application instructions for examples pertaining to various exclusion scenarios).
NRSA SCORING CRITERIA

**Overall Impact/Merit.** Reviewers will provide an overall impact/priority score to reflect their assessment of the likelihood that the fellowship will enhance the applicant’s potential for, and commitment to, a productive independent scientific research career in a health-related field, in consideration of the scored and additional review criteria (as applicable for the project proposed).

**Scored Review Criteria.** Reviewers will consider each of the five review criteria below in the determination of scientific and technical merit, and give a separate score for each.

- **Fellowship Applicant:** Are the applicant’s academic record and research experience of high quality? Does the applicant have the potential to develop as an independent and productive researcher in biomedical, behavioral or clinical science?
- **Sponsor(s), Collaborator(s), and Consultant(s):** Are the sponsor(s) research qualifications (including successful competition for research support) and track record of mentoring appropriate for the proposed fellowship? Are there (1) evidence of a match between the research interests of the applicant and the sponsor (including an understanding of the applicant’s research training needs) and (2) a demonstrated ability and commitment of the sponsor to assist in meeting these needs? Are the qualifications of any collaborator(s) and/or consultant(s), including their complementary expertise and previous experience in fostering the training of fellows, appropriate for the proposed research project?
- **Research Training Plan:** Is the proposed research plan of high scientific quality, and does it relate to the applicant’s training plan? Is the training plan consistent with the candidate’s stage of research development? Will the research training plan provide the applicant with individualized and supervised experiences that will develop research skills needed for his/her independent and productive research career?
- **Training Potential:** Does the proposed research training plan have the potential to provide the fellow with the requisite individualized and supervised experiences that will develop his/her research skills? Does the proposed research training have the potential to serve as a sound foundation that will lead the fellow to an independent and productive career?
- **Institutional Environment and Commitment to Training:** Are the research facilities, resources (e.g. equipment, laboratory space, computer time, subject populations), and training opportunities adequate and appropriate? Is the institutional environment for the scientific development of the applicant of high quality, and is there appropriate institutional commitment to fostering the fellows’ training as an independent and productive researcher?

As applicable for the project proposed, reviewers will consider the following additional terms in the determination of scientific and technical merit, but will not give separate scores for these items.

- **Additional Review Criteria.** As applicable, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an overall impact/priority score, but will not give separate scores for these items.
  - **Protections for Human Subjects:** the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation.
  - **Inclusion of Women, Minorities, and Children:** When the proposed project involves clinical research, the committee will evaluate the proposed plans for inclusion of minorities and members of both genders, as well as the inclusion of children.

Where applicable, the following sections will also be evaluated: Vertebrate Animals, Biohazards, Select Agent Research, Resource Sharing Plans.
INSTRUCTIONS FOR REFEREES

Reference Forms must be submitted to the eRA Commons* and may be submitted any time after the Funding Opportunity Announcement opens and **not later than the application receipt due date.** Your “letter of reference” **must** be submitted using the Fellowship Reference Form**. Failure to submit the required reference in the appropriate format may result in the application being returned to the applicant without review.

Please put the name of the applicant at the top of the form. The form has three sections: The first section is used to compare the applicant to other individuals of similar training and experience that you have known. The second section is used to enter your evaluation—Note that the form will automatically expand to an additional page as you enter your evaluation (in two pages or less, describe the applicant’s potential for a research career). The third section is the Referee information section. When you are finished with the Fellowship Reference Form, return to the eRA Commons page and complete the following required information:

- Referee First Name (Required)
- Referee Last Name (Required)
- Referee MI Name (middle initial) (Not Required)
- Referee E-mail (Required)
- Referee Institution/Affiliation (Required)
- Referee Department (Required)
- PD/PI (Fellowship applicant) Commons User ID (Required)
- PD/PI’s Last Name, as it appears on the PD/PI’s Commons account (Required) (will be validated to ensure they match)
- Funding Opportunity Announcement Number (Required and **must** match the number of the FOA under which the application is being submitted)
- Reference Letter Confirmation Number (for resubmissions only)
- Fellowship Reference Form – two pages maximum. Complete the format page using word processing software and then convert to PDF using PDF generating software**. Avoid scanning text attachments to convert to PDF since that causes problems for the agency handling the application.

Note that the Fellowship Reference Form can be submitted at any time prior to the receipt deadline. It is **not** necessary to wait until after the application is submitted before the Fellowship Reference Form is submitted; the two submissions are distinct. After you have submitted your Fellowship Reference Form, both you and the applicant will receive a confirmation of receipt by e-mail. Your e-mail confirmation will include a “Reference Letter” Confirmation Number. The Confirmation Number will be required when resubmitting reference forms. Please print the confirmation e-mail for your records.

* eRA Commons: https://commons.era.nih.gov/commons/reference/submitRefereeInformation.jsp
** Fellowship Reference Form: http://grants.nih.gov/grants/funding/424/416-1reference.doc
Tips on Writing National Research Service Award Predoctoral Fellowship Proposals From Real NRSA Reviewers

Greg J. Siegle
University of Pittsburgh School of Medicine
Sheri L. Johnson
University of California, Berkeley
D. Erik Everhart
East Carolina University
Tamara Newton
University of Louisville

Abstract
This is a collection of recommendations for writing National Research Service Award (NRSA) F31 predoctoral fellowship training grant proposals. These recommendations were generated by reviewers on the F12B study section devoted to Psychopathology, Developmental Disabilities, Stress and Aging to highlight features of the most successful applications we review as well as to address features that most frequently engender critical comments from reviewers. We have geared our comments specifically for predoctoral applicants applying via the F31 mechanism, but most of what we say also applies to the other NRSA awards (F30, F32).

Author Note
ADDRESS CORRESPONDENCE to Greg Siegle, Ph.D., Western Psychiatric Institute and Clinic, 3811 O’Hara St., Pittsburgh, PA 15213; e-mail: gsiegle@pitt.edu. Supported NIMH (K02 MH082998). Portions previously published in Siegle, G. J., & Ford, J. (2006). *Some hints on obtaining a K01 for post-docs and junior faculty*. Retrieved from [http://www.pitt.edu/~gsiegle/khints-wholeworld-071106.pdf](http://www.pitt.edu/~gsiegle/khints-wholeworld-071106.pdf), which was in part based on Ford, J. (2004, October). *Moving from Excellent (2.0) to Outstanding (1.0)*. Annual meeting of the Society for Psychophysiological Research, Santa Fe, NM; and Bartels, S., & Smith, G. (2004). Developing winning R01 services / interventions/neuroscience research proposals. *Geriatric Psychiatry Boot Camp*, July, Rochester, NY. We thank an NRSA reviewer who wished to remain anonymous as well as an anonymous reviewer of this manuscript for extremely helpful suggestions.
Tips on Writing National Research Service Award Predoctoral Fellowship Proposals From Real NRSA Reviewers

As National Research Service Award (NRSA) training grant (F31, F30, F32) reviewers on the F12B study section devoted to Psychopathology, Developmental Disabilities, Stress and Aging, we often see applications that conform to the “letter” of the program announcement but which receive suboptimal scores for common, preventable reasons that might be difficult to intuit before submission. Here we have assembled a collection of recommendations from NRSA reviewers that will hopefully address many of these considerations. A second goal is to highlight features of the most successful applications that we review. We have geared our comments specifically for predoctoral applicants applying for the F31 mechanism, but most of what we say also applies to the other NRSA mechanisms. There are, of course exceptions to most of what we have said below, and our points are probably best thought of as general characteristics of successful applications rather than hard and fast rules. Importantly, this is not an official document. It has neither been endorsed nor constructed by NIH representatives, program officers, review officers, or staff.

**Process**

*Read Up*

Visit the F kiosk, and read the program announcement and the guidelines for how F awards are evaluated. To get a feel for what reviews are like, read the sample F critique.

*Write the Application with your Mentor*

It is helpful to write the application with your mentor. It is easy to spot applications into which the mentor had little input, particularly if the applicant does not seem to know the field or the mentor’s work well enough. Make sure the mentor has read the application and has had time to comment on it before it goes out.

*Plan Ahead*

Most successful F31 grants are resubmissions. Our timeline for writing a K-award (faculty career award) may be a helpful guide in preparing to write your F31.

*Biosketch*

Use your personal statement for a *scientific* (not the rest of your life) biography of where you have been and what your professional aims are. Ideally, it should lead directly to the proposed project and from it.

In your personal statement, differentiate yourself from your sponsor--you should be working toward a career that is not exactly the same as the sponsor’s. It can have similarities, but the reviewers want to know you will not be a clone, and that you are capable of original ideas.

List in-press, submitted, and in-prep publications. Also list your presentations separately. For an F31, you should have at least one published or in-press publication and ideally more than two, with one first authorship to be competitive. If you do not have at least two publications, this is something that should be addressed in your sponsor’s letter, ideally with a plan for increasing publications. For an F32, having at least three papers is useful to be competitive.

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1 Online at http://grants1.nih.gov/training/F_files_nrsa.htm
5 Online at http://www.4researchers.org/articles/233
Listing your grades and GRE scores is important, so do not omit them. Telling the reviewers the percentiles for your scores is helpful. If your grades were terrible early in your undergraduate years, you might want your sponsor to note that your circumstances, motivation, or other factors have improved.

**Training**

*Career Development*

Take the career development sections seriously. Your sponsor should describe your career development plan in detail in the beginning and then you should describe it at the end under "goals for fellowship and training." Both should be multiple pages long and very explicit. The content can be virtually duplicated but from the perspectives of your advisor and you.

*Training Activities*

Proposed training should be above and beyond that which you would otherwise receive in your graduate program. Otherwise reviewers may think that you do not need the grant to get the proposed training. Think about specific skills that you need to go further in your career, such as advanced statistical expertise to help you with longitudinal or trials data, new ways of analyzing fMRI or psychophysiological data, training in psychopathology, and so on. If you are proposing to use a specific technology, such as fMRI, proposing to take a course or workshop in that technology--to either obtain or hone your skills as a scanning maven--can be very useful.

Ideally the proposed training should not deviate too far beyond the skills used in the research protocol. So, if you are going to associate coursework with the proposal, it is helpful to say how you will use it in your proposed research. It is rare that including much clinical work, teaching, or graduate courses unrelated to the research in a proposal would be perceived as helpful unless such activities clearly enhance the applicant's ability to complete the proposed research.

The ideal coursework is more than what you would already be gaining in your program, and enough that you can move ahead with your proposed research career. Both of these features should be noted. Proposed training should help you differentiate yourself from your sponsor so that you can have a different research career. So, if you are proposing to work with a clinical population and do not have explicit training and background with clinical populations, you should propose to get training in that clinical area--at least a course in the pathology (or if appropriate, psychopathology), and ideally spending time outside the research protocol with people with the pathology. Individual supervision should supplement your experiences learning about psychopathology. Experience in diagnostic interviewing seems to be particularly important for aspiring psychopathology researchers. Otherwise the committee may suggest you will be unqualified, at the end of the day, to do independent work with clinical populations. Be careful not to overstate what you will be able to do based on your level of training (e.g., if you are not a clinical psychologist, you should likely not be assigning diagnoses of schizophrenia). Unless your primary goal is to be a statistician, if you are proposing only retrospective data analysis for your primary research project it is useful to say that you will also have training experiences interacting with actual people; particularly, doing some relevant data collection, so that when you are done it is clear you can stand on your own.

It is useful to propose skills-building activities in manuscript and grant writing. Consider including an agenda for writing papers, attending conferences, and learning skills for grant writing. Bill Gerin's (2006) book, *Writing the NIH Grant Proposal: A Step-by-Step Guide* is good reading in this regard.
Choose your Sponsors and Consultants Carefully

It is helpful to choose a sponsor who is an expert in the research area of the proposal, has published a bunch and, ideally, has mentored other NRSA or K-awardees. If your sponsor does not have a strong track record of mentorship, it is useful to bring on a co-sponsor who does have a strong track record of mentorship. Reviewers will also expect the sponsor to have funding in the proposed research area. If the sponsor does not have that specific funding, or if the funding will end during your award period, it is worth commenting about how other existing funding (e.g., start-up funds or other mechanisms) can be used to support your work.

It is helpful for the sponsor to be local; ideally at your institution. If your sponsor is not at your institution, showing that you have a track record of working with your sponsor face to face despite location, particularly going regularly back and forth from the sponsor’s institution, can be helpful.

It is often helpful to have more consultants than just your sponsor. Ideally there should be consultants capable of advising on every aspect of your proposed work. For each sponsor/mentor/consultant you should say exactly what his or her unique (i.e., non-overlapping) contribution will be, and specify your specific involvement with each of them. For example, if you want to use a technology that your advisor has not used in published work, seek a consultant who will train you in those methods. If your project involves studying a form of psychopathology or comorbidity that your advisor is not expert in, seek a consultant to cover that area. All consultants should be well-published in their areas.

It is often helpful to have a statistician versed in your research area as a consultant. Having the statistician read the application before it goes out, and help with writing your analysis plan gets you lots of extra bonus points.

For applicants proposing to learn neuro-imaging, having a physicist and MR statistician on board as consultants can really help; ideally associated with the center where you are scanning.

Describe your Interactions with Sponsors and Consultants Explicitly

Meeting content and frequency with your sponsor, co-sponsor, and other consultants should be spelled out by them and by you--and these numbers should agree. A table of meetings is helpful to convey this information to the reviewer. Ideally, the primary sponsor will be available for weekly individual meetings. If a co-sponsor is at another site, provide a detailed plan not just for visiting, but how training will work during that visit. It is not sufficient to say “co-sponsor will be available by phone and web meetings.” Additionally, it is best to specify the types of readings that will be involved. Include specific training toward producing manuscripts and enhancing your grantsmanship. Finally, the sponsor and co-sponsor’s comments should be superlative if possible or at least strongly laudatory.

Research

Hit the Public Health Relevance Hard

How will the work you are doing help people? In other words, how will your research “translate” into improving public health-related issues? This is one strong feature on which the proposal will be judged. If you cannot answer it, neither can reviewers.

The aims should have a high likelihood of being informative; in other words, something that, if the study comes out as predicted, will lead to clinical understanding, new studies, or at least being cited by people in your field. This is particularly true for longitudinal studies, in which you want to include support for the idea that the changes you are proposing to examine over time have a high likelihood of occurring. For example, proposing a longitudinal study in which you examine how many people who are 12-13 years old develop hemophilia within one year of an
TIPS ON WRITING NRSA PROPOSALS

initial assessment may be deemed to have low likelihood of being informative due to the low base rate of hemophilia, and poor choice of a time-window in which the disorder will develop.

If you are examining a particular developmental period (e.g., puberty or old age) make sure to (a) clearly justify that period and (b) include relevant considerations for that developmental period. For example, if you are assessing children, will they be able to sit still for your assessments?

Use Your Training

The proposed work must represent a good training experience. You should not already have the proposed skills to do all the work you are proposing, and should emphasize what you will be learning from the proposed work.

The award is about you getting the training that will help your career to go in an interesting direction. The project is a chance to use that training. As such, it is useful to make sure that you are incorporating your training into your research plan. For example, if you propose to learn a statistical technique, include that technique in your proposed analyses.

Aims

It is often helpful to have no more than three specific aims (though applications with more can get a favorable review) and to fit them on one page. Reviewers want to see a simple story. This is not to say that the work will not have its complexities—rather, reviewers want to be able to understand the aims in a quick read. Remember, your application will be one of many they review, possibly at midnight after a long night of other grants. A confused reviewer can turn quickly into a grumpy reviewer, which does not bode well for scores.

The specific aims should not be dependent on each other; if hypotheses for Specific Aim 1 are not confirmed, it should still be useful to examine Specific Aim 2.

The aims should differentiate your work from your sponsor’s. In particular, they should not read just like those from a sponsor’s existing grant, as the differentiation from the sponsor may be questioned. If the overlap is large, make sure to include a statement that very clearly indicates what is new in your line of work compared to that of your sponsor.

Scope

Keep it feasible—more feasible than you think you have to. F’s are often hit for being “too ambitious.” In particular, proposing a reasonably powered RCT (randomized clinical trial) which is not piggy-backed on to a sponsor’s work is often considered too ambitious. You may want to consider a non-randomized pilot study instead. If you do propose an RCT it is helpful to have extensive documentation of feasibility and support from your mentors and consultants.

Fundamentals

Never neglect the fundamentals. Reviewers will evaluate the proposed work with regard to strength of methods, and they will look for signs that you kept the fundamentals of research at the forefront of your thoughts as you confronted the many challenges inherent in designing a feasible study.

If you are choosing measures from the literature, choose strong measures. Document their psychometric properties, including reliability and construct validity. This is particularly important for observational studies, where experimental manipulation may be difficult or impossible, and the soundness of your conclusions depends on the psychometric quality with which a construct is measured. Using a measure “because our lab has used it in the past” or “because my sponsor designed it” is not an acceptable rationale. The standard is currently lower for psychophysiology and imaging (i.e., no one reports psychometrics on these), and this can be
noted. Note--this recommendation should not preclude you from developing measures and tasks, but if you do, it is useful to propose to evaluate their psychometrics.

Address potential confounds. All research studies must grapple with potentially confounding variables, and reviewers know this. Explicitly identify potential confounds in your research, decide how to address them (e.g., randomization, exclusion, statistical covariates, etc.), and make your reasoning transparent.

Know the current and upcoming developments in your area. Most research areas, through cumulative efforts of multiple researchers, have developed state-of-the-science methods. Use them. And propose to get trained in them. A strong goal of the F31 mechanism is to help you to be an independent investigator in your area. If the methods you choose would only have made you a terrific investigator 10 years ago, reviewers may not support the application. Of particular note, for studies of emotion and information processing, it may be useful to include measures in addition to self-report (e.g., psychophysiology, eye-tracking, imaging, etc.).

Take care to not selectively report only the literature consistent with your hypotheses—a reviewer is bound to know of inconsistent studies if they exist—it is better to head these off at the pass than to rely on the ignorance of your reviewers.

Things to Include

Do include analytic plan and power-analysis sections. This should not be a toy or pilot data collection project—reviewers want to see that it will be publishable at the end of the day. Put in a time-line for what research activities will occur when.

Marketing

Say why the proposed training and research resources are essential to making you the scientist you want to be. This is above and beyond what you would be able to get or what is typically offered for your graduate program and also above and beyond what you would otherwise do for your dissertation research. One good answer here is that the money will protect your time for research so that you do not have to teach or spend time begging on the streets.

Make sure to say how your work will be funded. This is important because the F31 mechanism does not provide research funding. Particularly if it is fMRI, say where the resources for scanning will come from.

If English is not your first language or writing is not your forte, it is very helpful to have others read through and correct your grammar, spelling, and structure. Making the grant easier to read actually helps to get a positive review.

Responsible Conduct of Research

Take responsible conduct in research seriously. Training must be ongoing throughout the award, formal, and ideally not just online. Saying that you were trained in the past is not good enough. Providing details on course content as well as individual mentorship that will support ethics training is essential. Be specific about the frequency and duration of the training as these are explicit scoring criteria. Wherever possible, name the faculty members who will mentor you in training for responsible conduct in research, as well as the specific role(s) that they will play. Having the sponsor echo their roles in this regard can be helpful.

Human Subjects

Be careful in describing procedures you will use for protecting human subjects. Mistakes in following these conventions can be perceived as evidence that you are not being well-trained in the procedures in your field. If it is a clinical trial make sure to have a Data Safety and Monitoring Plan. If it has fMRI and there are women of child-bearing age, make sure to provide for pregnancy tests.
If you are working with a clinical population, discuss limits of confidentiality and referral mechanisms if needed, and consider a certificate of confidentiality if you are asking about illegal behaviors. If you are working with a procedure that has risks or discomforts, be honest about those. If you are gathering data online, be very specific about procedures you will use to protect electronic data.

Consultant Letters

It is useful to have letters from everyone remotely associated with your project. If you have a co-sponsor, it is important to have a letter from the co-sponsor. Not having letters can be interpreted as a lack of knowledge about the project or lack of involvement by the co-sponsor or consultant.

You may be asked to draft letters from your consultants and referees to you as well as your sponsor’s statement. Take care as you draft these letters. The letters are a big part of reviewers’ determination of your consultants’ belief in and commitment to you. Do not be modest--your consultants should know you, be enthusiastic about you and your project, and demonstrate that they are committing the proposed resources to help you.

Some thoughts as you review (or draft) consultant letters:
1. It is common for consultants to reiterate their understanding of their specific contributions to the applicant’s research and training plans in their letters of support.
2. If you are using a consultant or sponsor’s resources (e.g., their lab) it is useful for them to say they are on board with this use.
3. Chris Martin, Ph.D., has used the following sections in his letters: (a) involvement with mentee, (b) summary of mentee background, (c) mentee’s appropriateness for an F31, (d) correspondence of career development plan and research proposal, (d) endorsement of collaborators, (e) commitment of mentor’s resources, (f) description of mentor’s resources, and (g) support for mentee.
4. The NIMH guidelines for a career award reference letter are also helpful. They state that the letter involves an evaluation of the candidate with special reference to (a) potential for conducting research, (b) evidence of originality, (c) adequacy of scientific background, (d) quality of research endeavors or publications to date, (e) commitment to health-oriented research, and (f) need for further research experience and training.

Biosketch Personal Statements for Sponsors, Mentors, and Consultants

The NIH biosketch requires that sponsors, mentors, and consultants include a personal statement. It is helpful to write a draft of this section for them. Here is a template Siegle has used:

The proposed research involves.... I have expertise in all of these areas, including...., a long history investigating..., experience with..., and formative work in.... My work in this area began in.... I currently direct the...lab which is devoted to these themes. I have successfully administered major grants in this area and currently serve as PI or Co-I on multiple NIH grants using.... I have a strong track record of mentorship and co-mentorship of graduate students including NRSA's. Currently I mentor (number) graduate students and (number) post-doctoral fellows, of whom (number) have NRSA's. My students have regularly transitioned to prestigious post-doctoral and faculty appointments. I have and can provide the necessary resources to support (name)'s training goals.

Responding to Pink Sheets

1. Respond to every item in the review.
2. It is rarely useful to make changes on a revision that were not specifically identified as problems in the first submission, unless they were true design weaknesses.

3. If you get comments saying there is not enough methodological detail, be particularly careful to respond to these. If there are gross methodological lapses, it can be interpreted as a lack of mentor-involvement.

**Final thoughts**

This may seem like a lot of advice. Please don't let it dissuade you. The F series is a terrific and flexible award mechanism. The committees who review them are eager to see the next generation of researchers go on to brilliant careers and NIH is committed to using the F mechanism to help them do it. So good luck writing!
NIH Dissertation Awards (R36)

- Diversity theme ("broadening participation"): Targeted to women and/or underrepresented minorities; also to individuals with disabilities and/or disadvantaged backgrounds
- Support advanced graduate students during the research and writing periods of their doctoral studies
- Award amounts vary, but can be up to $100k+ over two years (NIDA)
- Must be US citizen or non-citizen national
- Application has six page limit

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Sample F32 Fellowship Proposal

PART I (Form Pages 1 to 6, 9)

Department of Health and Human Services
Public Health Service

Ruth L. Kirschstein National Research Service Award
Individual Fellowship Application

Follow instructions carefully.
Do not exceed character length restrictions indicated.

1. TITLE OF RESEARCH TRAINING PROPOSAL (Do not exceed 56 characters, including spaces and punctuation.)

Mechanistic Studies of NF-kB inhibition of Myogenesis

2. LEVEL OF FELLOWSHIP

F32

3. PROGRAM ANNOUNCEMENT/REQUEST FOR APPLICATIONS

NIAMS

4a. NAME OF APPLICANT (Last, First, Middle Initial)

Wang, Huating

4b. EMAIL ADDRESS

wang.522@osu.edu

4c. HIGHEST DEGREE

522

4d. PRESENT MAILING ADDRESS (Street, City, State, Zip Code)

4e. PERMANENT MAILING ADDRESS (Street, City, State, Zip Code)

4f. OFFICE TELEPHONE NO.

(406) 2600/Molecular Biology

4g. HOME TELEPHONE NO.

4h. PERMANENT PHONE NO.

4i. FAX NUMBER

5. TRAINING UNDER PROPOSED AWARD (See fields of training)

Discipline No.: 1000
Subcategory Name: 2600/Molecular Biology

7a. DATES OF PROPOSED AWARD

From (MM/DD/YY): 07/01/06
Through (MM/DD/YY): 06/30/09

7b. PROPOSED AWARD DURATION

In months: 36

8. DEGREE SOUGHT DURING PROPOSED AWARD

Degree:
Expected Completion Date:

9. HUMAN SUBJECTS

9a. RESEARCH EXEMPT

☑ NO ☐ YES

9b. HUMAN SUBJECTS ASSURANCE NO.

9c. NIH-DEFINED PHASE III CLINICAL TRIAL

☑ NO ☐ YES

10. VERTEBRATE ANIMALS

10a. ANIMAL WELFARE ASSURANCE NO.

11a. NAME OF SPONSOR (Last, First, Middle Initial)

Telephone:
Fac:
Email:

11b. NAME OF PROPOSED SPONSORING INSTITUTION

Research Foundation
The Ohio State University
1960 Kenny Road
Columbus, OH 43210

11c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

MVIMG/Human Cancer Genetics

11d. MAJOR SUBDIVISION

School of Medicine

12. ENTITY IDENTIFICATION NO.

DUNS NO.
1316401599A1
07-165-0709

13. NAME AND TEL. NO. OF ADVISOR IF DIFFERENT FROM 11a.

Telephone:
Fac:
Title:
Address:

14. NAME OF OFFICIAL IN BUSINESS OFFICE

Director
Health Sciences Office
B030 Graves Hall
333 West 10th Ave., Columbus, OH

15. APPLICANT CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and I agree to comply with the terms and conditions of award if an award is issued as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I certify that I have read the Ruth L. Kirschstein National Research Service Award Assurance, that I will abide by the Assurance if an award is made, and that the award will not support residency training.

SIGNATURE (Required of each applicant)

DATE

PHS 416-1 (Rev. 06/02)
**Kirschstein–NRSA Individual Fellowship Application**
(To be completed by applicant – follow PHS 416-1 instructions)

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**16. APPLICANT’S EDUCATION**  

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<td>Nanjing University</td>
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**17. APPLICANT’S TRAINING/EMPLOYMENT (After college)**

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**18. GOALS FOR KIRSCHSTEIN–NRSA FELLOWSHIP TRAINING AND CAREER**

The applicant's long-term career goal is to become an independent principle investigator in the future. The applicant has a strong background in molecular and cellular biology and is highly interested in the field of signaling transduction pathway and transcriptional regulation. NRSA fellowship will help the applicant move toward an independent, productive and satisfying career. The applicant plan to complete the proposed studies within two to three years and publish at least two top-quality papers.

**19. NAME AND DEGREE(S)**

Guttridge, Denis, C. Ph.D.

**20. POSITION/RANK**

Assistant Professor

**21. RESEARCH INTERESTS/AREAS**

Molecular Biology

**22. DESCRIPTION (Do not exceed space provided)**

NF-kappaB (NF-kB) is a transcription factor which plays a pivotal role in the regulation of cell growth, differentiation and cellular survival. Accumulating evidence suggest that NF-kB is involved in several skeletal muscle disorders although its mechanism of action is not well defined. In this proposal we attempt to gain insight into the roles of NF-kB in muscle diseases by understanding how NF-kB functions in skeletal myogenesis at a basic level. Recent studies have revealed that NF-kB plays an inhibitory role in skeletal myogenesis through multiple mechanisms. Based on our preliminary results we hypothesized that NF-kB inhibition of myogenesis could occur through direct or indirect repression of troponin gene expression. To test this hypothesis we propose to perform the following aims: 1) determine whether inhibition of troponin gene expression is mediated by direct NF-kB binding; 2) investigate whether NF-kB regulates troponin gene expression indirectly through YY1; 3) perform a genome-wide search of the YY1 and NF-kB target genes. Completion of these aims will provide us new mechanistic insights into how NF-kB function in skeletal myogenesis and advance our understanding of how it participates in the skeletal muscle disorders.
### Section 1 — Applicant

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### Section 2 — Sponsor

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### Section 3 — References (Minimum of 3)

(See instructions for submission of references.)

List full name, institution, and department of individuals submitting reference letters.

- Louis M. Mansky, Institute for Molecular Virology, University of Minnesota.
- Marshall V. Williams, Dept. of Molecular Virology, Immunology, and Medical Genetics, the Ohio State Univ.
- Deborah S. Parris, Dept. of Molecular Virology, Immunology, and Medical Genetics, The Ohio State Univ.
- Richard W. Burry, Dept. of Neuroscience, The Ohio State University

Other items: Personal Data Page for Fellowship Applicants

### Section 4 — Appendix

(3 collated sets. No page numbering necessary. Not to exceed 3 publications; 2 for predoctoral candidates.)

☐ Check if Appendix is included
**Kirschstein–NRSA Individual Fellowship Application**  
**Scholastic Performance**  
*(To be completed by applicant – follow PHS 416-1 instructions.)*

**NAME OF APPLICANT** *(Last, first, middle initial)*  
Wang, Huating

23. **SCHOLASTIC PERFORMANCE:**  
*Predoctoral applicants: List by institution and year all undergraduate and graduate courses with grades.*  
*Postdoctoral applicants: List by institution and year all undergraduate courses and graduate scientific and/or professional courses germane to the training sought under this award with grades. Complete block at bottom of page, if applicable. Senior applicants: Omit this page.*

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*Explain marking system if other than 1-100 or A, B, C, D, F. Show level required for passing. Predoctoral applicants state performance on Graduate Record Examination, if available.*
**Background**

(To be completed by applicant – follow PHS 416-1 instructions.)

<table>
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<th>PRIOR AND/OR CURRENT KIRSCHSTEIN–NRSA SUPPORT. List type (individual and/or institutional), level (pre or post), dates, and grant or award numbers.</th>
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**25a. ACADEMIC AND PROFESSIONAL HONORS. Include all scholarships, traineeships, fellowships, and development awards other than Kirschstein–NRSA. Indicate source of awards (NSF, Woodrow Wilson, etc.), dates, and grant or award numbers. List current professional societies, if applicable.**

1. 2004-2005 OSU Comprehensive Cancer Center Upon-on-roof Postdoctoral Fellowship
2. 2003 Participation Support Award for Symposium on Antiviral Drug Resistance
3. 2003 OSU Health Science Graduate and Postgraduate Research Day Award
4. 2002 Travel Fellowship for American Society for Virolgy Meeting
5. 2002 Professional Development Fellowship, Ohio State University
6. 2001 Travel Fellowship, MCDB Program, Ohio State University
7. 1998-2004 Graduate Research Assistantship, Ohio State University
8. 1996-1998 Outstanding Graduate Student Scholarship, Nanjing University
9. 1992-1996 Outstanding Undergraduate Student Scholarship, Nanjing University

**25b. TITLE(S) OF THESIS/DISSERTATION(S)**

Studies of Deltaretrovirus Assembly and Release

<table>
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<tr>
<td>Dr. Louis M. Mansky</td>
<td>Director</td>
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<td>Institute for Molecular Virolgy</td>
</tr>
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**27. APPLICATION FOR CONCURRENT SUPPORT**

[ ] NO  [ ] YES Using format below, list all support (training, research, supplies, travel, etc.) applied for that would run concurrently with the period covered by this application. Include the type, dates, source, and amount.

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PART I (Form Pages 1 to 6, 9)

Kirschstein–NRSA Individual Fellowship Application

Research

(To be completed by applicant – follow PHS 416-1 instructions.)

NAME OF APPLICANT (Last, first, middle initial)
Wang, Huating

28. RESEARCH EXPERIENCE
   a. Summary
   b. Doctoral Dissertation
   c. Publications (published, accepted, submitted, or in preparation)

29. REVISED APPLICATION

30. RESEARCH TRAINING PLAN
   a. Approximate percentage of proposed award time in activities identified below. (See instructions.)

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b. Research Training Proposal
c. Respective Contributions
d. Selection of Sponsor and Institution
e. Responsible Conduct of Research

See continuation page
28a Summary of Research Experience

A. 1998-2004 Graduate Research Assistant at Dr. Louis Mansky’s Lab

1. To study the bovine leukemia virus gag membrane targeting and late domain function. (funded by NIH grant 1R03AI053155-01). We have concluded that myristylation signal in the matrix (MA) domain of BLV Gag is required for membrane targeting and binding of BLV Gag. Mutations that disrupted the amino-terminal glycine residue (which would block the addition of myristic acid) leads to a drastic reduction in VLP production. We also identified that the PPPY domain within BLV MA was required for virus release. Mutation of the PPPY motif significantly reduced VLP production and this reduction was more severe in the presence of an active viral protease. These results were published on Journal of Virology 2002 and included in the doctoral dissertation.

2. To study the involvement of the matrix and nucleocapsid domains of the bovine leukemia virus Gag polyprotein precursor in viral RNA packaging. (funded by NIH grant 1K02AI057735-01). We have concluded that the MA and nucleocapsid (NC) domains are protein determinants of BLV RNA packaging. Mutagenesis of conserved basic residues as well as residues of the zinc-finger domains in the BLV NC domain of PrGag revealed residues led to a reduction in viral RNA packaging. These results were published on Journal of Virology 2003 and included in the doctoral dissertation.

3. To study the role of late domain in human T-cell leukemia virus type 1 particle release (NIH grant 7R03AI053155-03). We have concluded that both the PPPY and PTAP motifs in the C-terminus of HTLV-1 MA function as the L domain and influence virus release. These results were published on Journal of Virology 2004 and included in the doctoral dissertation.

4. To study the budding of HTLV-1 viral particles inside multi-vesicular bodies. (funded by NIH grant 7R03AI053155-03). We have concluded that HTLV-1 particles could assemble at both the plasma membrane and multivesicular bodies (MVBs) and PTAP motif directs the Gag to the plasma membrane. These results were submitted to Journal of Virology 2005 and included in the doctoral dissertation.

B. 2004-Present Postdoc Researcher at Dr. Denis Guttridge’s Lab
Comprehensive Cancer Center, The Ohio State University

To investigate the role of NF-kB in skeletal myogenesis. We have hypothesized that NF-kB inhibition of myogenesis could occur through the repression of troponin gene expression via direct binding of p65 to the troponin gene promoter or indirectly through YY1.
28b. Summary of Doctoral Dissertation

The assembly of retrovirus particles requires the expression of the Gag polyprotein precursor (PrGag), which is used as a principle building scaffold for retrovirus assembly and budding from infected cells. The retrovirus Gag polyprotein has all the necessary information to mediate intracellular transport to the cell membrane, to package full-length retroviral genomes, to direct assembly of virus particles, and to catalyze the final budding process. This dissertation was focused on the virus particle assembly of Deltaretroviruses, namely human T-cell leukemia virus type-1 (HTLV-1) and bovine leukemia virus (BLV). These viruses replicate to low titers in their natural hosts and are poorly infectious in cells culture. Information regarding the molecular details of their life cycles, including virus assembly, is limited. Virus-like particle (VLP) assay model systems were developed for BLV and HTLV-1 to test five hypotheses. The first hypothesis was that the myristylation signal in the matrix (MA) domain of BLV Gag was required for membrane targeting and binding of BLV Gag. Mutations that disrupted the amino-terminal glycine residue (which would block the addition of myristic acid) led to a drastic reduction in VLP production but did not eliminate Gag membrane localization, suggesting that other residues in Gag were involved in membrane targeting and binding. The second hypothesis was that the PPYY domain within BLV MA was required for virus release. Mutation of the PPYY motif significantly reduced VLP production and this reduction was more severe in the presence of an active viral protease. Examination of particles by electron microscopy revealed an abundance of particles that began to pinch off from the plasma membrane but were not completely released from the cell surface, indicating that the PPYY motif functions as a late domain (L domain). The third hypothesis tested was that the MA and nucleocapsid (NC) domains were protein determinants of BLV RNA packaging. Mutagenesis of conserved basic residues as well as residues of the zinc-finger domains in the BLV NC domain of PrGag revealed residues that led to a reduction in viral RNA packaging. Interestingly, when conserved basic residues in the BLV MA domain of PrGag were mutated to alanine or glycine, but not when mutated to another basic residue, reductions in viral RNA packaging were also observed. The ability of PrGag to be targeted to the cell membrane was not affected by these mutations in MA, indicating that these basic residues in the MA domain of PrGag influence RNA packaging, without influencing Gag membrane localization. It was further observed that i) a MA/NC double mutant had a more severe RNA packaging defect than either mutant alone, and ii) RNA packaging was not found to be associated with transient localization of Gag in the nucleus. These observations indicate that both the MA and NC domains of BLV Gag are involved in RNA packaging. The fourth hypothesis tested was that both the PPYY and PTAP motifs in the C-terminus of HTLV-1 MA function as the L domain and influence virus release. Mutation of either motif (i.e., PPYY changed to APPY or PTAP changed to PTRP) reduced budding efficiencies. Further analysis revealed that PPYY plays an essential role in HTLV-1 particle budding from the plasma membrane and could not be replaced by other late domain motifs, i.e. PTAP or YPDL, whereas the PTAP motif plays a subtler role in the virus release. The fifth hypothesis tested was that HTLV-1 particles could assemble at both the plasma membrane and multivesicular bodies (MVBs) and PTAP directs the Gag to the plasma membrane. I demonstrated that when HTLV-1 PTAP motif was altered, an accumulation of Gag proteins and virus particles were observed in intracellular compartments. These compartments were CD63-positive multivesicular bodies (MVBs). Further analysis excluded the possibility that these particles accumulated inside MVBs were a result of re-internalization of extracellular particles. It was further found that (i) the particle-containing MVBs traffic along microtubules using dynein-dynactin complexes recruited by RILP and these particles could therefore exit the cell by exocytosis. (ii) PI3P is likely to be a Gag receptor on MVBs as Inhibition of PI3K disrupted the MVB pathway.
28C. Publications (in chronological order)

A. Accepted Manuscripts


B. Manuscripts pending publication

C. Manuscripts in preparation
A. SPECIFIC AIMS

NF-kappaB (NF-κB) is a well-known transcription factor which plays a pivotal role in regulating innate and adaptive immunity. Accumulating evidence also suggests that NF-κB is involved in several skeletal muscle disorders including cachexia, disuse atrophy, and muscular dystrophies (14, 22, 36) although its mechanisms of action are not well defined. Insight into the role of NF-κB in muscle diseases can be gained by understanding how NF-κB functions in skeletal myogenesis at a basic level. Recent studies from several labs have revealed that NF-κB plays an inhibitory role in skeletal myogenesis through multiple mechanisms (1, 15, 16, 32, 50). Our preliminary results suggest that NF-κB inhibition of myogenesis could occur through repression of 'myofibrillar gene expression, including troponin gene expression. Results also indicate that silencing of troponin transcription might occur directly through NF-κB binding to the promoter or indirectly through the regulation of a repressor of myogenesis, YingYang 1 (YY1). Based on this data, we hypothesize that NF-κB inhibits skeletal myogenesis through the repression of troponin genes.

To test this hypothesis the following specific aims are proposed:

**Aim 1**: Determine whether inhibition of troponin gene expression is mediated by direct NF-κB binding.

1. Investigate whether Tnnt2 is repressed by NF-κB through direct binding.
2. Investigate whether Tnnt3 is repressed by NF-κB through direct binding.

**Aim 2**: Investigate whether NF-κB regulates troponin gene expression through YY1.

1. Determine whether YY1 is a NF-κB target gene.
2. 2. Determine whether Tnnt2 is a YY1 target gene.
3. Determine the mechanism of NF-κB regulation of troponin.

**Aim 3**: Perform a genome-wide search for the YY1 and NF-κB target genes.

1. Conduct a genome-wide screen of all the NF-κB and YY1 target genes by ChIP-on-chip technology.
2. Identify novel NF-κB and YY1 binding targets using a computational approach.

B. BACKGROUND AND SIGNIFICANCE

**Regulation of the skeletal myogenic program**

Specification and differentiation of skeletal muscle cells is driven by a family of muscle-restricted basic Helix-Loop-Helix (bHLH) transcription factors: Myf5, MyoD, myogenin, and MRF4 which are expressed in a hierarchical fashion during myogenesis (42). MyoD and Myf5 are expressed in myoblasts and act to establish the skeletal muscle lineage. Myogenin is expressed after myoblast fusion and required for the normal differentiation of the myoblasts established by the prior expression of Myf5 or MyoD. MRF4 is thought to be expressed only after muscle differentiation. These factors, in concert with members of the E2A and MEF2 families, activate the differentiation program by inducing the transcription of muscle specific genes such as myosin heavy chain (MyHC), Muscle creatine kinase (MCK), alpha actin (α-actin) and troponin isoforms.

Intensive investigation of the myogenic program has benefited from the use of in vitro cell culture models. The murine C2C12 cell line, which to a large degree faithfully recapitulates myogenesis during development and in regenerating muscle, has served as the standard cell culture model and has been instrumental for the identification and characterization of myogenic pathways. It is becoming increasingly clear that a complex network of both positive and negative signals is pivotal for skeletal myogenesis (42). Elucidation of how these signaling pathways function in skeletal myogenesis will advance our understanding of the role of these pathways in muscle disease.

**NF-κB signaling pathway and its role in skeletal myogenesis**

NF-κB belongs to the Rel family of transcription factors which regulates an exceptionally large number of genes, particularly those involved in immune and inflammatory responses (17, 25). Five mammalian Rel proteins have been identified, RelA (p65), c-Rel, RelB, p50 (NF-κB 1), and p52 (NF-κB 2) (Reviewed in (13, 47)). All of these proteins share a highly conserved 300-amino-acid Rel homology domain (RHD) in the amino-terminal half of the protein. NF-κB subunits are able to homo- or heterodimerize to form transcription factor complexes with a range of DNA-binding and activation potentials. Different NF-κB dimers exhibit different binding affinities for κB sites bearing the consensus sequence GGGRNNYYCC, where R is purine, Y is pyrimidine, and N is any base (37). Although all Rel members bind DNA, only RelA/p65, c-Rel, and RelB have
extended carboxy termini harboring a transactivation function. The most widely studied form of NF-κB is a heterodimer composed of p50 and p65 subunits which is a potent activator of gene transcription.

In most cells, NF-κB is found predominantly sequestered in the cytoplasm bound in an inactive complex with an IκB inhibitory protein family member. This family includes IκBα, IκBβ, IκBε, Bcl-3, p100 and p105. Upon stimulation, a rapid and transient activation of IκB kinase (IKK) occurs, leading to the phosphorylation of IκB, which targets IκB for ubiquitin-dependent degradation by the 26S proteasome complex, resulting in the liberation and nuclear translocation of NF-κB. NF-κB activation is induced by a wide variety of different stimuli including inflammatory cytokines such as TNFα and interleukin-1 (IL-1), bacterial lipopolysaccharide (LPS), viruses, UV light, and a variety of mitogens.

In addition to its well-established role in activating the transcription of genes involved in immunological responses, NF-κB also functions in the regulation of multiple cellular processes related to proliferation, adhesion, migration, and viability (6, 17, 25). The role of NF-κB in skeletal myogenesis has emerged in recent years. Previous studies from our lab and other groups have supported a negative role for NF-κB in myogenesis. We and others have found that proliferating myoblasts contain a relatively high constitutive level of NF-κB DNA binding and transcriptional activity, which decreases during differentiation (15, 32). Inhibition of NF-κB by various means was also seen to accelerate the myogenic program (1, 15, 32, 50). One mechanism by which NF-κB inhibits myogenesis is through the stimulation of cyclin D1, which maintains cells in a cycling state (15). Signaling activators of NF-κB such as TNFα (15), IL-1β (28), and the RIP homologue, RIP5 (40), also function as potent inhibitors of myogenesis. In the case of TNFα, inhibition of myogenesis occurs through the activation of NF-κB leading to the suppressed synthesis of MyoD (16). With respect to muscle disorders, it is becoming apparent that NF-κB is a key mediator of several muscle diseases including muscle cachexia (28), atrophies (20, 22) and dystrophies (38). Cytokines such as TNFα and IL-1 play a significant role in breakdown of skeletal muscle through the activation of the NF-κB signaling pathway (16, 27, 43).

YY1 and its association with skeletal myogenesis.

Our preliminary data suggested that one mechanism though which NF-κB regulates myogenesis is via YY1. YY1 (Yin-Yang1) is a zinc finger transcription factor that is highly conserved from Xenopus through humans. It is an abundant protein, and numerous potential target genes, both cellular and viral, have been identified (51). It can act either as a repressor, activator, or initiator of transcription. The functional diversity of YY1 depends on promoter context or interactions with many other coactivator, corepressor, or transcription factors. YY1 has been shown to physically interact with the histone acetyltransferases CBP and p300, the histone deacetylases HDAC1, HDAC2, and HDAC3, and the Arg-specific methyltransferase PRMT1 (3, 44, 56). In addition, YY1 interacts with various basal transcription factors, such as TATA binding protein, TFα, and IIIB (3, 12, 35, 52), and other transcriptional regulators, including Sp1, c-Myc, and C/EBPβ (2, 7, 29, 45, 46). YY1 recognizes a core 5'-CCATNNNT-3' CCAT box sequence flanked by more flexible nucleotides (21).

The role of YY1 in skeletal muscle differentiation is emerging as several myogenic genes were identified as YY1 target genes (11, 30, 31, 54). YY1 has been shown to act as a negative regulator of α-skeletal actin (30), muscle creatine kinase (MCK) and myosin heavy chain (MyHC) promoters (11, 54). It was proposed (11) that transcriptional repression of the MCK enhancer and the MyHC promoter in proliferating myoblasts was regulated by YY1 binding and subsequent recruitment of a complex containing both Ezh2 and HDAC1 that silences transcription through histone methylation and deacetylation. During skeletal myogenesis, the YY1/Ezh2/HDAC repressive complex is removed from the MCK and MyHC promoter and replaced by activator SRF and the MyoD family of transcription factors, along with associated acetyltransferases to activate myogenic gene expression.

YY1 has recently been shown to be involved in human heart failure through its repression of the human alpha myosin heavy chain (alpha MyHC) promoter (48). It was also reported that IL-1β can induce a novel form of cardiac myocyte hypertrophy characterized by the repression of α-actin or β-MyHC gene expression. Interestingly, this repression by IL-1β may be due, at least in part, to the activation of YY1 (41). Despite the numerous YY1 target genes identified, little is known about the regulation of YY1 gene. Further understanding of the mechanisms underlying the regulation of YY1 and its regulation of muscle genes might provide insights into muscle differentiation in both development and muscle diseases.
Significance. NF-κB signaling pathway has been associated with multiple diseases including rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, asthma and cancer (6, 34, 53). In recent years there has been increasing evidence suggesting that NF-κB plays a role in skeletal muscle disorders (4, 5, 19, 20, 38). A better mechanistic elucidation of NF-κB’s role in regulation of normal muscle development will facilitate our understanding of the requirement and function of NF-κB signaling in muscle disease and eventually lead to new therapeutic treatments. The studies proposed here will lead us to a novel mechanism underlying the inhibitory role of NF-κB in skeletal myogenesis and will provide information that may prove useful in elucidating how the NF-κB signaling functions in muscle disease or in differentiation of other muscle or non-muscle lineage.

C. PRELIMINARY RESULTS

NF-κB negatively regulates troponin gene expression in proliferating myoblasts.

Previous studies from our lab have suggested that NF-κB represses myogenesis through multiple mechanisms (15, 16, 27). To identify genes that are regulated by NF-κB in proliferating myoblasts and gain further insight into the mechanisms by which NF-κB might function to repress muscle differentiation, microarray analysis was performed in proliferating C2C12 vector control myoblasts or myoblasts stably expressing a mutant form of the IκBα inhibitor of NF-κB, IκBα super repressor (IκBα-SR). IκBα-SR is mutated at serines 32 and 36, and as such is longer subject to phosphorylation and subsequent proteasome degradation following an NF-κB-activating stimulus, therefore functions as a potent and specific inhibitor of NF-κB activity(9). IκBα-SR expressing myoblasts are devoid of nuclear p50/p50 and p50/p65 complexes, and are unresponsive to TNF-α stimulation. Myogenesis is also rapidly accelerated in these cells compared to vector control myoblasts (15).

Microarray analysis was used to identify differentially regulated genes that were elevated in IκBα-SR over that of vector control myoblasts. Of the 284 genes that showed differential expression of 4 fold or higher, some of the highest ratios were observed with troponin isoforms, troponin C (TnnC)(25.8 fold), troponin T (TnnT)(12.8 fold), and troponin I (TnnI)(10 fold) (Table 1), suggesting a repressive mechanism of NF-κB on troponin gene expression in proliferating myoblasts. Although increases in MyHC (8.9 fold) and actin (Actn)(10 fold) was also observed in IκBα-SR cells, other hallmark markers of myogenesis such as myogenin, MEF2 transcription factors, or sarcomeric genes tropomyosin (Tpm), actinin, and nebulin were not elevated, suggesting that NF-κB regulation of troponin might be specific.

To confirm the results from microarray analysis, RT-PCR and real-time PCR were performed. Consistent with the microarray analysis, the results showed an increased level of troponin isoforms, MyHC and α-actin but not tropomyosin gene expression in proliferating C212 IκBα-SR myoblasts (Figure 1A and B). Furthermore, luciferase reporter assay showed the promoter activity of Tnn2 gene was elevated in the C212 IκBα-SR myoblasts (Figure 1C). These data suggest a potential mechanism of NF-κB inhibition of myogenesis by selectively repressing the expression of some of the myofibrillar genes including troponin. In the following sections, we will use the troponin gene as a model for our mechanistic studies.

Repression of the troponin promoter by direct binding of NF-κB.

Figure 1. Expression of troponin isoforms is increased in IκBα-SR C212 myoblasts. RT-PCR (A) and real-time PCR (B) analyses of sarcomeric genes expressed in vector and IκBα-SR expressing C212 myoblasts. C. Reporter activity of Tnl-Luc in vector and IκBα-SR cells.
Repression of troponin by NF-κB could occur through several mechanisms. An appealing model is that NF-κB binds directly to the gene promoter to inhibit its transcription (Figure 2A). Although NF-κB is not recognized as a direct transcriptional repressor, data has shown that in response to UV or chemotherapy, activation of NF-κB leads to the transcriptional suppression of anti-apoptotic genes Bcl-XL and c-IAP (10). Repression of the Bcl-XL promoter by p65 occurs through the recruitment of the HDAC co-repressor. In an alternative model NF-κB could function indirectly by regulating the expression of a transcriptional repressor of troponin (Figure 2B).

We began by testing the first model of NF-κB binding directly to the troponin promoter to inhibit its transcription. Focus was placed on fast skeletal muscle troponin I gene (Tnni2) promoter since its promoter region has been well characterized and comparisons of Tnni2 from several species revealed 5 conserved 10-bp motifs with similarity to consensus NF-κB binding sites, GGGRNNYYCC, in the proximal promoter region (referred to as sites A-E, Figure 3A) (39).

EMSA analysis was performed to test if NF-κB can bind to these motifs in vitro. Radiolabeled probes containing these elements were used in EMSA analysis with nuclear extracts from C2C12 myoblasts either untreated or treated with TNFα for 15 minutes. An oligonucleotide derived from the major histocompatibility complex (MHC I) promoter (GGGATTCCCCC) was used as a positive control. Results showed that MHC I formed two complexes with a typical migration pattern of the classical p65/p50 heterodimers and p50/p50 homodimers (Figure 4A). The levels of the complexes increase when cells were treated with TNFα indicating the NF-κB activation by TNFα. No obvious complexes formation was observed with oligonucleotides B, D, and E, but complexes were formed with oligonucleotides A and C, which migrated to a similar extent as the MHC I probe (Figure 4A). Supershift EMSAs were performed to test if these complexes contained p65 or p50 proteins. The results showed the complexes formed with MHC I were supershifted to a higher position by p65 or p50 antibodies whereas no supershifts occurred with oligonucleotides A or C (Figure 4B), suggesting that NF-κB is most likely not a component of these higher-molecular-weight complexes.

In addition to the Tnni2 gene, we are also interested in testing the direct binding model on Tnnt3 which was significantly elevated in hBo-SR myoblasts (Figure 1). Scanning of Tnnt3 promoter identified a putative NF-κB binding site, GGGGATTCCC at -79. Whether NF-κB can bind to this site or not will be examined in proposed EXPERIMENT DESIGN AND METHODS for Aim 1.
YY1 is regulated by p65 at the transcriptional level.

The above data indicated that NF-κB inhibition of troponin gene expression is likely to occur indirectly through a downstream factor that could be a transcriptional repressor of the troponin promoter (Figure 1E). In an attempt to identify this intermediate, a second microarray analysis was performed to identify genes positively regulated by the p65 subunit of NF-κB. RNAs isolated from p65 wild type or null MEFs treated with TNFα for 1hr and RNA was used in this array analysis. Many known NF-κB target genes were upregulated in the wild type cells such as TNFα, lxBα and IL-6 (Table 2). Interestingly, YY1, a negative regulator of several muscle genes, was also upregulated in the wild type line (fold change 4.4). We therefore speculated that p65, through transcriptional control of YY1, may be involved in repression of troponin gene expression.

To test the idea that YY1 is an NF-κB target gene, C2C12 myoblasts were treated with TNFα for different periods of time (0, 1, 2 and 4hr) and YY1 expression was monitored by real-time RT-PCR. As a positive control, the induction of lxBα, a bona fide NF-κB target gene, was monitored next to YY1. The results showed that the TNFα treatment induced both lxBα and YY1 expression (Figure 5A). Levels of YY1 and lxBα transcripts increased at 1hr and remained elevated at 4hrs. To determine if regulation of YY1 was p65 dependent, TNFα treatment was performed with p65+/+ and p65−/− MEFs. Results showed an evident induction of YY1 in p65+/+ cells while no induction was observed in p65−/− cells arguing that the TNFα induction of YY1 was through p65 (Figure 5B).

Regulation of YY1 gene expression via p65 suggested that p65 may be a direct transcriptional regulator of this gene, we thus initiated a search for possible NF-κB binding sites on YY1 promoter. Scanning of the mouse YY1 promoter revealed two short motifs with similarities to consensus NF-κB binding sites, which are referred to as sites, A (GGGGGGCCCCC) and B (GGAGGACCCT), at positions −170 and −153 from the transcriptional start site (Figure 6A). Radiolabeled oligonucleotides containing these sequences were therefore used in EMSA analysis with nuclear extracts from C2C12 myoblasts either untreated or treated with TNFα. Results showed that NF-κB complexes were bound to the A site, which could be supershifted with p65 and p50 but not with IgG (Figure 6B). The binding was abolished when site A was mutated to TTGGGCCCCAA. No binding activity was detected with a probe for the B binding site, suggesting that site A is likely to be a NF-κB binding site on the YY1 promoter. Experiments proposed in Aim 2 will further explore the mechanism through which NF-κB regulates YY1 using a number of complementary approaches.

Figure 6. Direct binding of p65 to the YY1 promoter. (A). Schematic illustration of YY1 promoter containing two putative NF-κB binding sites and a Sp1 binding site. (B). EMSAs. C2C12 myoblasts were either untreated (+) or treated (−) with TNFα for 15 min and EMSA performed with radiolabeled probes containing putative YY1-A, YY1-B or YY1-A-mutant NF-κB binding sites. Complexes formed were identified by supershift EMSA with either antisera specific to p65 or IgG. Arrows denote p65 and p50-containing complexes and supershift complexes (SSI and SSIII).
Tnni2 is a YY1 target gene.

Since the above results suggested that YY1 is positively regulated by p65, we speculated that the p65 inhibition of troponin gene expression might occur through YY1. This is an appealing model, given that YY1 has already been shown to repress myofibrillar genes, such as α-actin and MyHC, which were also shown in Figure 1 to be repressed target genes of NF-κB. To test this hypothesis we determined whether YY1 was able to bind to the troponin promoter. We chose to study Tnni2 since this promoter did not appear to be directly bound by p65, thus making it an attractive YY1 target gene. We examined the Tnni2 promoter and enhancer region located within the first intron for putative YY1 binding sites. YY1 has a loose consensus binding site, with the 5'CATATG core sequence being essential for binding (21). Careful inspection of the mouse Tnni2 promoter and enhancer revealed several important regulatory elements including consensus MEF-2, E-box, CCAC and CAGG box elements (Figure 6A). Three CCAT boxes were identified in the enhancer region of Tnni2 with site A closest to the consensus YY1 binding sequence: (C/G) (G/T/A) CCATNTTN. Oligonucleotides were generated from the putative binding site A as well as from the MyHC promoter, used as a positive control (11). EMSAs were performed with the nuclear extracts from proliferating C2C12 myoblasts. Results showed a complex formed with the MyHC promoter (Figure 7B lane 1) which was supershifted with YY1 antibody (lane 2). Consistent with previous findings (11), YY1 binding to the MyHC promoter diminished in differentiated myotubes (lanes 3 and 4). EMSA results further revealed the formation of a complex bound to the putative YY1 site A in the Tnni2 enhancer (Figure 7B lane 5). This complex was also supershifted with YY1 antibody (lane 6) and like the MyHC promoter, was reduced in myotubes (lanes 7 and 8). In comparison, no complex was detected when the CCAT box was mutated to GGAT (lane 9-12). These results indicate the possibility that Tnni2 is a direct target gene of YY1.

To assess the in vivo association of YY1 on the Tnni2 enhancer, we performed ChIP assays in proliferating C2C12 myoblasts with YY1 antibodies and analyzed the immunoprecipitated DNA fragments by PCR with specific primers for three different regions of Tnni2 enhancer A, B and C. Region A contains the putative YY1 binding sequence tested in EMSA, while regions B and C contain three additional putative CCAT boxes (Figure 6D). YY1 was found to associate with the chromatin regulatory region A, but not regions B and C (Figure 6D lanes 3, 4 and 5). These results therefore indicate that YY1 inhibits Tnni2 expression through binding to its enhancer region.

D. EXPERIMENTAL DESIGN AND METHODS

Aim 1. Determine whether Inhibition of troponin gene expression is mediated by direct NF-κB binding.

The goal of this aim is to investigate the possibility that NF-κB represses troponin gene transcription through direct binding to the troponin promoter. We will confirm our results on Tnni2 and then extend the studies to Tnni3 gene, which by scanning analysis also revealed a consensus NF-κB site at position -79 with respect to the transcription starting site.
1.1. Although our preliminary data obtained from in vitro EMSAs suggest that p65 may not directly bind to Tnnt2 promoter, the following experiments will be performed to substantiate our argument.

In vivo ChIP assay. Preliminary data in this proposal demonstrated our ability to perform ChIP assays in C2C12 myoblasts and myotubes. ChIP assays will be conducted on C2C12 cells treated with or without TNFα using a p65 antibody that has been previously shown to be effective in ChIP analysis in mouse cells. As a positive control, known NF-κB target promoters such as IκBα and IL-6 will be included in the assays. A control with IgG immunoprecipitation and amplification of a random region within the Tnnt2 gene will be included as negative controls. If there is direct binding of p65 on the Tnnt2 promoter, an amplification of a fragment will be expected from the p65 IP.

Functionality assay. The Tnnis luciferase plasmid used in preliminary study (Figure 1C) will be used as the parental construct. Several mutant luciferase reporter constructs will be generated with site-directed mutations in the putative NF-κB binding site. Wild type and mutant reporter plasmids will be transfected in C2C12 myoblasts. The next day, cells will be treated with TNFα for various periods of time before a standard reporter assay is performed with cell lysates. If the repression of Tnnt2 is through sites A to E, we expect that TNFα treatment should repress the activity of the wild type reporter but not the mutant reporter. To validate these findings, reporter assays will be performed with wild type and mutant Tnnt2 reporter plasmids in p65+/+ and p65/- MEFs transfected with MyoD. If repression does occur through this site, we would expect to observe higher mutant reporter activity in the p65+/+ compared to p65/- fibroblasts. Through these studies we will be able to determine whether NF-κB repression of Tnnt2 occurs through direct binding.

1.2. In this aim we will focus on Tnnt3 which showed highly elevated expression in C2C12 SR cells. The initial scanning of this gene has identified a strong consensus NF-κB binding site in its promoter. We will thus test the hypothesis: NF-κB repressesTnnt3 through direct binding to its promoter. Similar to the Tnnt2 study, we will first use the EMSAs and ChIP assays to determine whether p65 binds to the Tnnt3 promoter via the putative binding site. Luciferase assays combined with a mutagenesis approach will then be used to validate the functionality of this site.

Analysis of p65-Tnnt3 binding. First, Radiolabeled probes containing the putative NF-κB binding site, GGGGCATTCC, will be used in EMSAs analysis with nuclear extracts from C2C12 myoblasts either untreated or treated with TNFα for 15 minutes. Oligonucleotides derived from the MHC I promoter will be used as a positive control. Supershifting will be performed with antibodies against p65 or p50. If NF-κB can bind to this site directly, complex formation with Tnnt3 oligos will be expected. These complexes should display a same migration pattern as MHC I-containing complex and should be supershifted by p65 or p50. Similarly, a mutant probe will be generated and tested for its ability to bind to NF-κB. In vivo ChIP assay will then be performed to confirm the in vitro results. ChIP assays will be conducted on C2C12 cells treated with or without TNFα using the p65 antibody as described above.

The repression of p65 on Tnnt3 promoter activity. We will also generate a luciferase reporter containing -800~ +100 of the promoter sequence of Tnnt3 fused to a luciferase gene. The reporter will be transfected into C2C12 myoblasts followed by treatment with TNFα. Reporter assays will be performed with the cell lysates. If NF-κB represses Tnnt3 through binding to its promoter, a decreased reporter activity with TNFα treatment will be expected. We will then mutate the putative binding site and perform the same experiment. To test if the repression is through the p65 subunit, reporter assays will be performed with wild type and mutant Tnnt3 reporter plasmids in p65+/+ and p65/- MEFs. If repression is specific to p65 subunit, we would expect to observe higher reporter activity in the p65+/+ compared to p65/- fibroblasts.

Aim 2. Elucidate the mechanisms by which NF-κB represses Troponin expression through YY1.

The focus of this aim is to dissect two regulations: First, the up-regulation of YY1 gene expression by p65. Second, the repression of troponin gene expression by YY1. Establishment of these two regulations will lead us to the second model proposed in Figure2B: NF-κB inhibits troponin gene expression through YY1.
2.1. We will begin by testing the first part of the hypothesis: YY1 is a potential novel NF-κB regulatory gene. Several complementary criteria will be employed to validate that NF-κB is a positive regulator of YY1.

1. YY1 expression can be induced by NF-κB activators.
2. Loss of NF-κB leads to loss of YY1 expression.
3. NF-κB regulates YY1 promoter activity.
4. NF-κB binds to YY1 promoter in vitro and in vivo.

Real-time RT-PCR will be used to establish whether YY1 meets the first two criteria. Luciferase reporter assays will be used to determine the third criteria. Finally, a combination of EMSA and ChIP assays will be used to determine whether YY1 meets the last criteria and identify the DNA elements recognized by NF-κB.

Expression analysis of YY1 gene. Our preliminary data showed that YY1 expression can be induced by TNFα in C2C12 cells and that this induction is p65-dependent. To further support this result, two other potent activators of NF-κB, IL-1β and LPS will be used to treat C2C12 cells for various times (0, 1, 2, and 4hr) followed by RNA extraction from the cells. Real-time PCR will be performed to detect the levels of YY1 transcripts. If YY1 is transcriptionally upregulated by NF-κB, increased levels of YY1 RNA will be expected with the treatment of these inducers. To address the cell-specificity of the induction, similar experiments will be performed in a non-muscle cell line, such as 10T1/2 fibroblasts. To determine the specificity of YY1 regulation with respect to the p65 subunit, we will use MEF cells deficient in different NF-κB subunits, including p65-/-, p50-/-, p52-/-, c-rel-/- MEFs. These cell lines are currently available in our laboratory (18). Real-time PCR will be performed to determine YY1 RNA expression. If the NF-κB mediated induction is regulated via the p65 subunit, we will expect no induction of YY1 in p65-/- MEFs compared with other null cell lines.

Next, we will use the loss-of-function approach to further determine the positive regulation of YY1 by p65. First, siRNA against p65 will be used to delete the p65 expression in C2C12 cells and YY1 RNA and protein levels will be assessed by real-time PCR and Western blot respectively. A decreased level of YY1 RNA and protein is expected. Second we will examine the amount of YY1 RNA and protein in p65 wild type and null MEF cells. A decreased level of YY1 mRNA and protein will be expected in null MEF cells lacking p65 activity.

Lastly, to substantiate the in vitro results, we will examine YY1 RNA and protein levels in p65 wild type and null mice which are also available in the laboratory (18). RNA and protein will be isolated from different types of muscles such as tibialis anterior, gastrocnemius, and quadriceps from p65 wild type and null mice followed by real-time RT-PCR and Western blot analysis respectively. Additionally, muscles will be fixed and stained with YY1 antibody and examined using immunofluorescence microscope.

Effect of p65 expression on YY1 promoter activity. To test the transcriptional regulation of p65 on YY1 promoter activity, we will perform reporter assays with the YY1 promoter fused to a luciferase reporter. The parental construct will contain -800 to +60 of YY1 promoter. The reporter will be transfected into C2C12 myoblasts in the presence or absence of a p65-expression plasmid, pCMV-p65 which has been exhaustively used in our laboratory (15, 16). If the YY1 promoter is positively regulated by p65, an increase in luciferase activity will be observed with the co-expression of p65. We will then mutate the putative NF-κB binding site which we identified in the preliminary data (Figure 6) and transfect mutant reporter in C2C12 myoblasts in the presence or absence of a p65 expression plasmid. If this site is indeed the p65 binding, the mutant reporter activity should not increase with the p65 co-expression. To strengthen this analysis, cells will be transfected with wild type or a mutant YY1 reporter and then treated with NF-κB inducers, such as TNFα, IL-1, and LPS. Reporter activity will then be determined 1hr and 6hr post-treatment. Wild type but not mutant YY1 reporter activity is expected to be elevated by the inducing agents.

Analysis of p65-YY1 promoter binding. In the preliminary studies, in vitro EMSA analysis suggested that NF-κB was able to bind a putative NF-κB binding site within the YY1 promoter (Figure 6). To substantiate this binding activity in vivo, ChIP assays will be performed in proliferating myoblasts. Chromatins from C2C12 cells will be immunoprecipitated with p65 antibody. Primers will be designed to amplify the region encompassing the putative NF-κB binding site. Negative controls including immunoprecipitations with IgG and the amplification of sequence distal to the NF-κB binding site in the YY1 promoter will be included. The amplification of a known NF-κB binding target such as iκBα promoter will be used as a positive control. ChIP assays will also be performed in vector and iκBα-SR expressing myoblasts. If NF-κB is associated with the YY1 promoter in vivo, then this association would be expected to be inhibited in iκBα-SR cells devoid of NF-κB activity. To further
2.2. The combination of expression analysis, reporter assay, EMSA and ChIP analysis should be sufficient to elucidate whether p65 transcriptionally regulates YY1 through a NF-κB binding site on the YY1 promoter. The second part of the hypothesis is to establish a link between YY1 and Tnni2. Although YY1 is known to repress several muscle-related genes, the interaction of YY1 with the Tnni2 promoter or enhancer has not been reported. The preliminary data in this proposal suggest that YY1 is able to bind to a putative YY1 binding site on the Tnni2 enhancer (Figure 7). This part of the aim will focus on determining if YY1 represses the Tnni2 gene expression through binding to its promoter. Similar assays used to establish YY1 as a NF-κB target gene will be used to test whether Tnni2 is an YY1 target gene.

**Effect of YY1 expression on Tnni2 promoter.** Mouse Tnni2 enhancer region containing the YY1 binding site will be fused to the luciferase reporter. The reporter plasmid will be transfected into C2C12 cells along with different amounts of an YY1 expression plasmid, pCMV-HA-YY1, which we have obtained from Dr. Shi Yang's laboratory at Harvard University (49). If YY1 represses Tnni2 transcriptional activity, a dose-dependent decrease of Tnni2 reporter will be expected. If this is found to be the case, a mutant Tnni2 reporter plasmid with a mutation in the YY1 binding site will be constructed and further used in reporter assays. We would predict that the mutant reporter will not react to YY1 overexpression.

**Binding of YY1 on Tnni2 promoter.** The association of YY1 with Tnni2 promoter has been demonstrated by EMSAs and ChIP assays in our preliminary data (Figure 7). Based on these results we will next determine the functionality of this association during myogenesis. It was proposed that YY1 recruits a co-repressor complex containing Ezh2, HDAC1 to the promoter of MyoD to inhibit its expression in myoblasts. The repressive complex is then replaced by an active complex containing MyoD and acetyltransferase PCAF and CBP/p300 at the onset of differentiation (11). To test if the same model applies to the Tnni2 gene regulation, we will use ChIP assays to examine the association of a panel of repressors and activators, including HDAC1, Ezh2 (repressors), and MyoD, PCAF, and CBP/p300 (activators), with the Tnni2 enhancer in either myoblasts or myotubes. We envision that in proliferating myoblasts, the binding of YY1 on the Tnni2 regulatory region will lead to the recruitment of a repressive complex containing Ezh2 and HDAC1 to remodel chromatin in a closed conformation thereby inhibiting Tnni2 transcription. We further predict that at the onset of differentiation, this co-repressor complex will be replaced by the transcriptional activators MyoD, PCAF and CBP/p300 to remodel chromatin in an open conformation to activate Tnni2 expression. Based on these predictions, we will expect to observe the association of YY1, Ezh2 and HDAC1 with the Tnni2 enhancer in the myoblasts but lost in myotubes. In contrast, the association of MyoD and CBP/p300 would be expected only in myotubes. To confirm the function of the complex, the methylation status of histone H3 will be analyzed by ChIP using antibodies against trimethyl-Histone H3 (Lys27) and acetyl-Histone H3 (Lys9). Methylated histone H3 is expected in myoblasts, characteristic of a closed chromatin complex, while acetylated histone H3 is expected in myotubes, representative of an open chromatin complex. All the antibodies mentioned above are commercially available and have been used in ChIP assays by other groups (11).

In the final part of this analysis, we will attempt to link the two parts of the hypothesis together by seeking to demonstrate whether NF-κB can regulate YY1, which in turn will bind to the Tnni2 promoter to repress its expression. This will be tested in C2C12 vector and IxBα-SR myoblasts. We will first examine the amount of YY1 RNA and protein in C2C12 vector or IxBα-SR cells. If our model is correct, a decreased level of YY1 mRNA and protein is expected in C2C12 IxBα-SR cells lacking p65 activity. We will also examine the association of YY1, HDAC1, MyoD and CBP/p300 to the Tnni2 enhancer region using ChIP assay. We will expect to observe a decreased binding of YY1 and HDAC1 and increased binding of MyoD and CBP/p300 to the Tnni2 regulatory region in C2C12 IxBα-SR cells which lack NF-κB activity and have lower levels of YY1. This would correlate well with the elevated Tnni2 expression in IxBα-SR cells shown in the preliminary data (Figure 1) and argue that NF-κB regulation of YY1 is critical to maintain silencing of the Tnni2 gene. The argument will be strengthened by expanding the analysis on several other muscle genes including MyHC and α-actin which have been previously identified as YY1 target genes (11, 30, 31) and shown to be NF-κB repressed genes in our preliminary study (Figure 1). Through these studies we will be able to test a novel
mechanism through which NF-κB inhibits skeletal myogenesis, whereby positive regulation of YY1 by NF-κB causes the subsequent repression of myofibrillar genes.

**Alm 3. Genome-wide search of the YY1 and NF-κB target genes.**

Since several tropinin and other muscle myofibrillar genes were found elevated in myoblasts lacking NF-κB activity, it suggests that NF-κB repression of myofibrillar genes may be a general mechanism to inhibit myogenesis. Studies from Aim 1 and Aim 2 will identify two possible mechanisms underlying the repression of Tnni2 and Tnnt3 genes by NF-κB. It will be interesting to test whether similar mechanisms function for other tropinin and sarcomeric genes. To identify all the YY1 target promoters and NF-κB binding promoters we propose to perform a genome-wide promoter screen using integrated computational genomics and ChIP-on-chip technology in collaboration with Dr. Tim Huang and Dr. Ramana Davuluri.

3.1. ChIP-on-chip is a modern technique which combines chromatin immunoprecipitation (ChIP) with promoter DNA microarrays, allowing the identification of direct transcription factor binding on a genome-wide scale. Unlike gene expression arrays or genetic experiments that ascribe a role for regulators in a process but cannot distinguish direct from secondary effects, this method allows us to define the direct interactions between regulators and targets (8). Dr. Tim Huang, an associate professor in our department, is an expert in this technology. On the other hand, computational approaches harness the power of analyzing vast amounts of complex genomic data, and when used in combination with data gathered by ChIP-on-chip, have facilitated the identification of transcriptional binding and led to the elucidation of regulatory networks (8). Dr. Ramana Davuluri is a principal investigator in our department and an expert in computational genomics. Both laboratories are located across the hall from our own laboratory. Collaboration efforts between the laboratories of Dr. Huang and Dr. Davuluri have resulted in the successful identification of the estrogen receptor a target genes (23, 24, 33). We have had several informal discussions related to the genome wide promoter screen of NF-κB regulated genes and believe it is feasible to carry out this aim.

In order to perform ChIP-on-chip, we will utilize a CpG island microarray which is commercially available from the Microarray Centre at the University Health Network, Canada (http://www.microarrays.ca) (23). CpG islands often correspond to promoter regions and provide a reliable tool for promoter prediction. Coupling CpG island arrays with ChIP is proven to be a high-throughput method for the identification of in vivo target promoters (55). Proliferating C2C12 vector and hκBα-SR cells will be cross-linked followed by the standard ChIP protocol using antibodies specific for YY1 or p65. Immunoprecipitated DNA will be purified and labeled with the fluorescence dye Cys-5. Total input DNA will be labeled with Cys-3 dye. Both fluorescence dyes are available from Amersham, Buckinghamshire, UK and the DNA-labeling system can be purchased from Invitrogen. Parallel assays with nonspecific IgG antibody or no antibody will be included as controls. Equal amounts of Cy5-labeled immunoprecipitation products and Cy3-labeled input DNA samples will be cohybridized onto CpG island array slides which contains 7298 GC-rich promoter sequences. We are confident that we will be able to perform ChIP-on-chip assays because our laboratory has already demonstrated the ability to perform ChIP and microarray assays respectively.

3.2. The analysis of microarray data will be assisted by Dr. Davuluri’s group to identify the NF-κB and YY1 target genes. Because the array elements range from 0.5 to 2kb in size, the ChIP-on-chip assay will not pinpoint the precise position of NF-κB or YY1 binding. Therefore, additional analysis to locate these sites is needed. This will be performed by using the MATCH program in the TRANSFAC database (http://www.gene-regulation.com) as well as by the MatScan program that was designed by Dr. Davuluri and his research group. If putative NF-κB or YY1 binding sites are identified by this integrative approach, a number of genetic and biochemical approaches as described in Aim 1 and 2 will be used to validate the results. These studies will be exciting because the results will give us a global picture of YY1 and NF-κB target promoters and extend our analysis beyond the Tnni2 and Tnnt3 genes.

**Summary.** Together, the studies proposed here will provide a mechanistic insight into the role of NF-κB in inhibiting myogenesis. Based on preliminary data, we hypothesize that inhibition of myogenesis can be achieved by repressing tropinin transcription directly or indirectly. The proposed aims will allow us to investigate the indirect repression through YY1 and also identify the possible direct binding target promoters. Results from these findings will help elucidate the function of NF-κB in skeletal myogenesis and may provide insight into how this transcription factor contributes to skeletal muscle disorders.


**STASTICAL ANALYSIS**

Results will be expressed as mean plus or minus SEM. Statistical analysis will be performed using the Student's t test and the Wilcoxon rank sum test. P values less than 0.05 will be considered to be significant.

**VERTEBRATE ANIMALS**

1. **Proposed use of animals**: Mice deficient in p65 knockout strains will be used to determine the regulation of YY1 by p65 in vivo. Muscles samples from resulting progeny will be isolated and used for biochemical studies. Although it is not anticipated, mice that lose weight (>20% of body weight), stop eating, or become diseased or incapacitated will be euthanized. It is anticipated that up to 10 mice will be used.

2. **Justification**: The use of knockout mice will allow us to confirm the cell culture results in vivo. The minimum number of animals will be used to provide statistically significant data.

3. **Veterinary Care**: All animals will be housed in an AALC approved facility (Wiseman Hall) according to AVMA and NIH/USDA guidelines. All animal research will be conducted using appropriate biosafety facilities.
and practices approved by protocols of the OSU, IACUC, protocol number 01A0083 (reviewed 07/01/05). Certified laboratory animal veterinarians provide animal care at all sites.

4. **Euthanasia:** All mice will be euthanized by inhalation of 100% CO2. Early removal criteria: Mice that lose weight (>20%), are lethargic, emaciated, paresis/paralysis, hunched posture, ruffled haircoat, or lameness will be euthanized.
30C. Respective Contributions
Once I was able to obtain sufficient preliminary data, Dr. Guttridge and I collectively decided that I was ready to apply for my own funding. We began to discuss the grant, first informally, and then with more structure and substance in our monthly meetings. I judged these meetings as being highly collaborative, where Dr. Guttridge would instruct me on how to formulate my specific aims, and I would provide insight in the design of the experiments. Dr. Guttridge also made suggestions regarding potential collaborations that could be initiated with the Davuluri and Huang group, and I have been able to initiate studies with several members of these groups. These combined efforts were also exhibited during the writing portion of my proposal. Although I wrote the application on my own, Dr. Guttridge provided me with valuable input that helped me better understand the rationale of my experiments and possible future studies that can be developed from this research project.

30D. Selection of Sponsor and Institution
The applicant studied the life cycle of Human T-cell leukemia virus type 1 (HTLV-1) during her graduate training. T-cell transformation by HTLV-I involves deregulation of cellular transcription factors, including members of the NF-kappaB (NF-kB) family. During the course of her graduate training, the applicant gained strong interest in NF-kB signaling transduction pathway and transcriptional regulation in a host cell system and decided to broaden her horizons by entering into the NF-kB field after obtaining the Ph.D.

For personal reason, the applicant has to remain at OSU for her postdoctoral training. The current sponsor, Dr. Denis Guttridge’s lab, is specialized in investigating the role of NF-kB signaling pathway in cell cycle and cell differentiation. Dr. Guttridge, who possesses an excellent research record, has extensive knowledge and expertise on the field of NF-kB signaling transduction. His laboratory is middle-sized with a combination of two postdocs and four graduate students, which provides the applicant an opportunity for independent learning as well as mentoring graduate students. The laboratory possesses a high-quality and dynamic research and emphasizes on collaboration in between lab members and with outside labs. Therefore, it makes an ideal environment for the applicant to make a transition from the field of Virology to the field of signaling transduction pathway and transcriptional regulation. At the same time, the applicant’s expertise on molecular and cellular biology will contribute significantly to the on-going research in Dr. Guttridge’s laboratory. Shortly after joining the lab, the applicant was working together with other labmates and made a significant contribution to a Cancer Cell paper published recently.

The research proposed by the applicant in this application integrates genetic and biochemical techniques as well as high-throughput genomic methods and computational approaches to gain insight into the mechanism underlying a transcriptional regulatory event. It provides a unique training opportunity for the applicant to learn cutting-edge knowledge and new technologies in the field of transcriptional regulation. Furthermore, since the proposed studies will involve two other laboratories, the applicant will learn how to collaborate with intramural laboratories.
The Ohio State University
Associate Vice President for Research
Trudy E. Cushen

12/05/2005

has successfully completed the Responsible Conduct of Research web-training on

Huating Wang

Certificate of Completion

Responsible Conduct of Research

Responsible Research Practices

The Office of OIO
PART II (Form Pages 7 to 9) Continued

Name of Applicant (Last, first, middle): Wang, Huating

31. BIOGRAPHICAL SKETCH
Provide the following information for the sponsor (co-sponsor). DO NOT EXCEED FOUR PAGES.

NAME OF SPONSOR (CO-SPONSOR):
Denis. C. Guttridge

POSITION TITLE
Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
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<tr>
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<td>1986</td>
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<td>Ph.D.</td>
<td>1996</td>
<td>Mol. Genetics</td>
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<tr>
<td>University of North Carolina, Chapel Hill</td>
<td>Postdoctoral</td>
<td>1996-2001</td>
<td>Mol. Biology</td>
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A. Positions and Honors

Positions and Employment

1986-1988 Graduate Student, California State University; Research on Artemia Hemoglobin. Mentor, R. Acey
1990-1996 Graduate Student, University of California, Irvine; Research on molecular characterization of PN-1 gene expression. Mentor, D. Cunningham
2001-present Assistant Professor, Division of Human Cancer Genetics and Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University

Honors and Awards

1996-1998 UNC Lineberger Cancer Center Postdoctoral Fellowship
1998-2000 American Cancer Society Postdoctoral Fellowship
2000-2001 Cancer Research Institute Postdoctoral Fellowship
2000 Keystone Symposia Award
2001 Joseph S. Pagano Postdoctoral Fellow Research Award
2002 V foundation Scholar Award

B. Peer-Reviewed Publications


C. Research Support

ONGOING

Guttridge (PI)
NIH/NCI
K01 CA97953-02
Cytokines and NF-kappa B Regulation of Cancer Cachexia
The major goal of this project is to investigate the role of inflammatory cytokines TNFα and IFNγ in cachexia and how signaling to NFκB regulates fat and skeletal muscle integrity.

Guttridge (PI)
NIH/NCI
R01 CA098466-01
NF-kappa B Regulation of Muscle Wasting Cancer Cachexia
The major goal of this project is to focus on determining whether NF-kB is involved in cancer-induced muscle wasting, and if so, to define the mechanisms of this regulation.

COMPLETED RESEARCH

Guttridge (PI)
Ohio Cancer Research Associates
NF-kappa B Regulation of Cell Growth Control in G1/S
7/1/02-6/30/04

Guttridge (PI)
ACS institutional seed grant
Cytokines Regulation of Adipogenesis in Cancer Cachexia
10/01/01-09/31/02
Kirschstein-NRSA Individual Fellowship Application

Facilities and Commitment

(To be completed by sponsor – follow PHS 416-1 instructions.)

NAME OF APPLICANT (Last, first, middle initial)
Wang, Huating

32. Identify the research and research training support available to the sponsor and the applicant during period of proposed award.

NIH K01 CA97953  Cytokines and NFkB Regulation of Cancer Cachexia  Guttridge, PI  3/20/02-2/28/07
total directs: $704,455

NIH R01 CA098466  NFkB Regulation of Muscle Wasting in Cancer Cachexia  Guttridge, PI  3/07/03-2/28/08
total directs: $831,250

NIH AR052787 NFkB/IKK Signaling in Myogenesis and Muscle Disease  Guttridge, PI  2006-2011
Pending

33. SPONSOR'S PREVIOUS FELLOWS/TRAINEES
Give total number of pre- and postdoctoral individuals and provide information on a representative five. List their present employing organizations and position titles or occupations.

NA - see continuation page

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FACILITIES AND COMMITMENT STATEMENT

in the space below and on continuation pages, complete the following items. Identify each item by number and title.

34. Training Plan, Environment, Research Facilities.
Describe the research training plan for the applicant, include such items as classes, seminars, and opportunities for interaction with other groups and scientists. Describe the research environment and available research facilities and equipment. Include information that will help reviewing groups evaluate the applicant and the proposed training. Indicate the relationship of the proposed research training to the applicant's career.

35. Number of Fellows/Trainees to be Supervised During the Fellowship. Indicate Pre-or Postdoctoral.

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

37. Human Subjects/Vertebrate Animals Use and Description.

See continuation pages.

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38. CERTIFICATION: We, the undersigned, certify that the statements herein are true, complete, and accurate to the best of our knowledge. If this application results in an award, appropriate training, adequate facilities, and supervision will be provided, and we accept the obligation to comply with the Public Health Service terms and conditions of award. We are aware that any false, fictitious, or fraudulent statement or claim may subject us to criminal, civil, or administrative penalties.

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<td>Denis C. Guttridge, Ph.D.</td>
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OFFICIAL SIGNING FOR SPONSORING INSTITUTION

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<td>kim C. Carter</td>
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34. Training Plan, Environment and Research Facilities

Although Huating is the first postdoctoral fellow from my laboratory that is applying for research funding, I feel strongly that the research plan that has been formulated provides Huating the opportunity to succeed and reach her career goal as a principal investigator and leader of a research group. This plan is structured at two levels. The first occurs in my laboratory where Huating has been given several project concepts that, with her technical and intellectual skills she obtained as a graduate student, can be molded into her personal research study. In Huating’s case, she was provided with microarray data that steered her into several new directions by which NF-κB might negatively regulate skeletal myogenesis. In just a little over one year’s time since she arrived in the laboratory, Huating obtained some very strong preliminary results to identify YY1 as a novel NF-κB target gene, and she is continuing to pursue the prospect that NF-κB might repress myocardial gene expression through direct binding of their promoters. As part of the development of her research study, I meet with Huating on a monthly basis to go over her progress and share ideas with her as to the direction of her studies. She also presents Powerpoint style updates of her research every 6 months in our monthly laboratory groups meetings, which provides us with further opportunity to discuss the direction of her projects and her thinking for future independent studies. In addition, the research plan in my laboratory attempts to stimulate as much communication and scientific complementation without creating scientific overlap and personal conflicts. To implement this part of the plan, I have Huating working across the bench with one of the graduate students, Nadine Bakkar, to give her the opportunity to communicate and gain experiences in training that will be helpful to her when she begins her own laboratory. This also benefits the graduate students to learn techniques and develop their own sense of scientific independence. This particular partnership between Huating and Nadine has worked out very well, as each will be a co-author on their respective manuscripts relating to NF-κB and myogenesis.

The second aspect of the research plan is the experience that Huating will obtain through interactions in our department. The department of Molecular Virology, Immunology, and Medical Genetics, as its name implies, is quite diverse scientifically. One of our weekly seminar series is in house, where PIs from the department present their latest studies. This environment is highly favorable for a postdoctoral fellow to remain scientifically well balanced. In addition, the department has a weekly Research in Progress seminar series where postdoctoral fellows and graduate students present their work in a formal seminar style. The presentations are limited in time to provide an ample question and answer session that is a real benefit to the speakers as a training vehicle. As a former Up on the Roof Postdoctoral fellow, which is a prestigious departmental fellowship awarded to only two new fellows yearly, Huating participates in the recruiting of new postdocs. This allows her to engage in further scientific discussions with future peers and to potentially develop early professional relationships. Huating also has at her disposal at the university experts in various fields of skeletal muscle biology, ranging from development, physiology, and disease. I am also in the process of developing a skeletal muscle research group at Ohio State University and nearby Children’s Research Institute whose aim is to forge closer interactions among PIs, postdoctoral, and graduate students interested in multiple aspects of muscle biology.

The research environment is highly conducive for Huating’s postdoctoral training. As you will read in her proposal, part of her studies will involve bioinformatics and ChIP on CHIP technologies to identify novel NF-κB transcriptional targets in muscle cells. For these studies she will be working closely with other postdoctoral fellows in the laboratories of Drs. Davuluri and Huang, who are experts in these respective technologies (see attached letters). Huating will also have at her disposal a rich environment of equipment resources. The laboratory itself is 900 sq ft and equipped for molecular, protein, and recombinant DNA work, including a gel documentation system. A tissue culture room, common liquid nitrogen containers, radioactive room, freezers, ice machine, luminometer, and a Real Time PCR cycler, are located on the same floor. A dark room, cold room, and shared equipment supported by the department and the Human Cancer Genetics Program are easily accessible on adjacent floors. The laboratory maintains two G4 Macintosh and two Pentium IV Dell PC computers along with two HP laser printers that are available for the laboratory personnel. Additional common PC computers are located on our floor and adjacent floors in the department and in the Human Cancer
Genetics Program, with available scanners and laser printers. All the computers are networked and connected to the main server at the College of Medicine at Ohio State University. The department also operates a Genotyping-Sequencing Unit and an Affymetrix and custom array core on the 4th floor of Wiseman Hall, adjacent to our building. A Real Time PCR core, under the direction of the Ohio State University Comprehensive Cancer Center, is located on the third floor of the building. A Flow cytometry core and an imaging core containing a confocal microscope are located across the street from our building in the Heart and Lung Research Institute.

As described above, the research training that will Huating receive places her in a favorable position to help her meet her career goals as an independent investigator. In this training, she will have the possibility of learning the latest technologies through her collaborations with Drs. Davuluri and Huang, and will develop new skills in animal handling, through her interest to study YY1 regulation in skeletal muscle during embryogenesis and post natal mouse development. She will also be exposed to multiple experts in skeletal muscle, which should help her as she transitions from a postdoctoral position to a principal investigator and a leader of her own research group.

35. Currently supervised Fellows and Trainees
In my research, I currently supervise two fellows including Huating and four predoctoral trainees. My other postdoctoral fellow, Dr. Jingxin Wang, has been in the laboratory for two years, and has a second co-author MCB paper and is currently in the process of submitting his first, first author manuscript. Of the graduate students, two are senior members in their fourth years in the laboratory. One, Swarnali Acharyya, has a first author manuscript in JCI and Cancer Cell, has presented seminars in the Human Cancer Genetics Program and in the Research in Progress seminar series, attended an international muscle meeting in Canada, and is currently applying for her own predoctoral fellowships. The other, Erin Hertlein, has a first author in MCB, has also presented seminars in the Human Cancer Genetics Program, took one of two first place prizes in our 2004 Graduate Student Presentation Series (total of 15 presenters), and attended an NF-κB Keystone symposia. My third student, Nadine Bakkar, has spent two years in the laboratory and has a first author manuscript in Signal Transduction, and co-authored in Erin’s MCB paper. She is currently preparing to submit her second first author manuscript on NF-κB and myogenesis, which Huating will also appear as second author. My fourth student, Jason Dalhman, has just completed his first year in the laboratory, but is currently focused on NF-κB mechanisms of cell growth control and is working in partnership with my fellow, Jingxin Wang. The research group in the laboratory is highly communicative, spirited, and works in a team like atmosphere, which I believe are important components for a successful training environment.

36. Qualifications of the Candidate and Potential for a Research Career
When Huating Wang applied for our departmental Up on the Roof Fellowship program, I attended her interview seminar. What stuck me about her talk is how confident she was of her science and she portrayed a real sense of independent thinking. This latter quality really shined through in the question and answer session of her seminar, where she was able to field with ease some challenging questions from the faculty and other Up on the Roof Fellows. With these personal qualities and her scientific accomplishments, which included multiple first author quality papers, there is no doubt that Huating could have landed a top postdoctoral fellowship position outside Ohio State where she earned her doctorate degree. For personal reasons, she decided to remain at Ohio State, and I was extremely pleased when she decided to join our research group. In the short time that Huating has been in my laboratory, it is evident that she possesses many of the necessary qualities that I believe are needed to reach the next level of her career as an independent investigator. I have already mentioned her confidence and ability to think independently. She also has a real knack to learning new techniques. Although CHIP assays are widely used now, Huating developed this technique for our laboratory and is teaching others, such as Nadine, how to perform this assay for her studies. She has also been able to develop skills in bioinformatics to search for NF-κB binding sites in myofibrillar promoters. I have much confidence that she be successful in her experiments that she proposed in her research proposal, including the CHIP on CHIP assays. Furthermore, I have been very impressed in her writing style, and it should be mentioned that she wrote her proposal with very little input from myself or other members of the laboratory. I
believe the one area that Huating will need improvement on is her communication skills and teamwork attitude. These attributes are very important for a successful scientist to have, and the postdoctoral fellowship position is the best time in a career that this can be developed. The research plan that I discuss above for Huating should help her to refine these skills. The fact that she made the decision on her own to ask all the members of the laboratory to read and comment on her proposal tells me that she is well on her way to obtaining these additional skills.

37. Vertebrate Animal Use
1. Proposed use of animals: Mice deficient in p65 knockout strains will be used to determine the regulation of YY1 by p65 in vivo. Muscles samples from resulting progeny will be isolated and used for biochemical studies. Although it is not anticipated, mice that lose weight (>20% of body weight), stop eating, or become diseased or incapacitated will be euthanized. It is anticipated that up to 10 mice will be used.
2. Justification: The use of knockout mice will allow us to confirm the cell culture results in vivo. The minimum number of animals will be used to provide statistically significant data.
3. Veterinary Care: All animals will be housed in an AALC approved facility (Wiseman Hall) according to AVMA and NIH/USDA guidelines. All animal research will be conducted using appropriate biosafety facilities and practices approved by protocols of the OSU, IACUC, protocol number 01A0083 (reviewed 07/01/05). Certified laboratory animal veterinarians provide animal care at all sites.
4. Euthanasia: All mice will be euthanized by inhalation of 100% CO2. Early removal criteria: Mice that lose weight (>20%), are lethargic, emaciated, paresis/paralysis, hunched posture, ruffled haircoat, or lameness will be euthanized.