



Preparing an NIH grant in 2010

Susan A. Slaughaupt, Ph.D.
Associate Professor of Neurology
Center for Human Genetic Research



What are the Major Changes in Application Forms?

Highlights:

- Shortened page limits
- Application reorganized to align with review criteria
- New “Research Strategy” section
- Biosketch limits publications; includes personal statement re: ability to do the research
- Facilities and Resources focuses on aspects that specifically contribute to accomplishment of the research



Goals of Restructured Applications

Aligns structure and content of the forms with review criteria

Focuses applicants and reviewers on the same elements

Ensures a more efficient and transparent review process

Goals of Shortened Page Limits

Reduces burden / maximize reviewer time

Focuses on the essentials of the science

Emphasizes impact

Avoids information overload

Shorter Page Limit Guide

Section of Application	Page Limits
Introduction for Resubmission Application	1
Specific Aims	1
Research Strategy: R03, R13/U13, R21, R36, R41, R43, Fellowships (F), SC2, SC3	6
Research Strategy: R01, single project U01, R10, R15, R18, U18, R33, R24, R34, U34, R42, R44, DP3, G08, G11, G13, UH2, UH3, SC1	12
Biographical Sketch	4

* Page limits may vary for other funding mechanisms.

Check Funding Opportunity Announcement: http://enhancing-peer-review.nih.gov/page_limits.html



Reviewers Benefit from Shorter Applications

- **Old Practice**

- Too much focus on how to “do” the research
- Significant mentoring on how to revise
- Long, detailed application/too much to read

- **New Focus**

- Impact: Is research worth doing
- Clear signal via criteria whether to resubmit
- Streamlined applications (easier to validate, less to read)



Preparing a Grant – a practical guide

- **“Administrative” sections of the grant**
 - **Due to research management 15 business days before grant deadline**
 - **Form Pages (including project summary and relevance, key personnel, performance sites, etc.)**
 - **Biosketch (for all key personnel)**
 - **Budget**
 - **Budget justification**
 - **Resources**
 - **Vertebrate animals section**
 - **Human subjects section**



Preparing a Grant – A Practical Guide

- **Science sections of the grant:**
 - **Due to research management 5 business days before due date**

 - **Introduction (1 page) (resubmissions only)**
 - **Specific aims (1 page)**
 - **Research Strategy (12 pages)**
 - **Significance, Innovation, Approach**
 - **Bibliography**
 - **Multiple PI leadership plan (if applicable)**
 - **Letters of support**
 - **Resource sharing plan**



Step by Step:

- 1. Get the directions and FOLLOW THEM!!!**

<http://grants.nih.gov/grants/funding/phs398/phs398.html>

Read the relevant sections and think about what they are asking for!



Project Summary and Relevance

- **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.**
- **Relevance – in two or 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.**

Biosketch

- ★ • **Personal Statement:** why your experience and qualifications make you particularly well-suited for your role in the project
- **Positions and Honors:** List in chronological order
- ★ • **Publications:** NIH encourages applicants to limit the publications to no more than 15. Do not include submitted or in prep. You may choose to include publications based on recency, importance to the field, or relevance to the proposed research
 - NOTE instructions on the Public Access Policy, be sure the PMID numbers or the NIHMS numbers are given
- **Research Support:** List both ongoing and completed research projects for the past three years. Begin with projects most relevant to the research proposed. **DO NOT** include effort or costs.
 - Note: Research Support (highlights accomplishments) **is not** Other Support (reports on funding available)



Budget and Justification

- **Modular vs. detailed budget??**
- **Modular capped at 250K direct per year**
- **Prior approval required for direct costs > 500K in ANY year**

- **Personnel: provide brief background, expertise, justification for inclusion, what they will do**
- **Provide true justification for costs, don't be afraid to ask for what the research will actually cost.**



Resources

- **Identify the facilities to be used (laboratory, clinical, animal, computer, office, other). If appropriate, indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Describe only those resources that are directly applicable to the proposed work.★**
- ★ • **Describe how the scientific environment in which the research will be done contributes to the probability of success (e.g., institutional support, physical resources, and intellectual rapport). In describing the scientific environment in which the work will be done, discuss ways in which the proposed studies will benefit from unique features of the scientific environment or subject populations or will employ useful collaborative arrangements.**
- **If there are multiple performance sites, describe the resources available at each site.**



Resources



For Early Stage Investigators: describe institutional investment in the success of the investigator, e.g., resources for classes, travel, training; collegial support such as career enrichment programs, assistance and guidance in the supervision of trainees involved with the ESIs project, and availability of organized peer groups; logistical support such as administrative management and oversight and best practices training; and financial support such as protected time for research with salary support.



Introduction

- **Limited to one page ★**
- **Resubmissions only – NIH will only accept one resubmission ★**
- **summarizes the substantial additions, deletions and changes to the application. It must also include a response to the issues and criticism raised in the Summary Statement.**
- **The substantial scientific changes must be marked in the text of the application by bracketing, indenting, or changing typography. Do not underline or shade the changes. If the changes are so extensive that essentially all of the text would be marked, explain this in the Introduction.**



Specific Aims

- **Limited to 1 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.**
- **Single most important page in the grant – spend a lot of time on it!!!**



Example:

The overall goal of this grant is to determine why blondes have more fun. While countless studies have been performed, we still don't understand the pathogenesis.... this is a very important problem.....our preliminary data is very exciting and shows....Given what we have accomplished to date, we are now poised to determine exactly why blondes have more fun..... Specifically, we aim:

1. To
2. To.....
3. To.....

Well-written, strong introductory paragraph that sets up the need for your work followed by detailed Aims. Your aims should be 'obvious' after reading Introductory paragraph.



Research Strategy – 12 pages

- **Significance**
- **Innovation**
- **Approach**
- **You may address SIA for each specific aim individually, or you may address SIA for all aims collectively**
- Vast majority of PIs address collectively:
 - Significance (2 pages)
 - Innovation (1 page)
 - Approach (9 pages, includes progress/preliminary data (labelled) and then Approach for Aim 1, 2, 3, etc.



Significance

- **Importance of the problem or the critical barrier to progress in the field**
- **How will the proposed project improve scientific knowledge, technical capability, and/or clinical practice**
- **Describe how the concepts, methods, technologies, treatments, services or interventions that drive the field will be changed *if the proposed aims are achieved.***



Innovation

- **Explain how the application challenges and seeks to shift current research or clinical practice paradigms**
- **Describe any novel theoretical concepts, approaches, or methodologies, instrumentation or interventions to be developed, and advantages over existing**
- **Explain any refinements, improvements, or new applications of theoretical concepts approaches, instrumentation, or interventions.**
- **NOT ALL APPLICATIONS HAVE TO BE INNOVATIVE (although I wouldn't leave this section blank!!)**



Approach

- **Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims**
- **Anticipated Difficulties & Alternative Strategies**
 - **Very important section of the grant that many people do not address**
 - **Opportunity to conduct a direct dialogue with the reviewers & show your sophistication**
 - **Should have been preparing as you go along by anticipating 'potholes' in the road and designing around them; now is the time to show it**
 - **Also deal with what happens if you get stuck on a point; it happens to everyone - just show you've thought it out**
 - **Use a Q&A format just as they would; put the hard questions to yourself and answer them**



Preliminary Studies/Progress Report

- **Preliminary Studies (new grants) or Progress Report (renewals) must be included as part of the Research Strategy, keeping within the three sections: significance, innovation, approach**

A Note on Formatting – Do you want to read this???

Specific Aim #2: To characterize the in vitro nature of mutations in the PROK2/PROKR2 pathway found in humans with GnRH deficiency. Hypothesis 3: Mutations in both the ligand and receptor in the prokineticin 2 pathway causing human GnRH deficiency will represent loss of function mutations. Hypothesis 4: A subset of mutations in PROKR2 will produce differential defects on downstream signaling pathways that will refine our understanding of the structure-function of PROKR2 receptor signaling in GnRH neurons.

Overview of our Approach to SAs #1 & 2: Our Preliminary Results provide clear evidence that the spectrum of reproductive disorders associated with mutations in the prokineticin system, even in this small number of early patients, is broad and complex. They occur in both sexes, involve defects at multiple levels of the reproductive axis (i.e., hypothalamic and gonadal), and likely involve important non-reproductive defects such as circadian rhythm abnormalities. Hence, detailed phenotyping and genotyping of patients with mutations in this system will be important. We have clear preliminary data that these DNA sequence changes result in loss of function in vitro. Consequently, we will approach SAs #1 & 2 in a coordinated fashion that involves 6 distinct steps: 1) Determine all DNA sequence changes representing putative mutations in PROK2 and PROKR2 in our cohort of >900 probands with defects in the GnRH pacemaker and well-phenotyped NIH/KS/AHH; 2) Characterize the in vitro biologic activity of each of these changes; classify it as a loss/gain of function; and determine its in vitro severity; 3) Examine each mutation in at least 2 separate second messenger signaling assays to characterize its impact in each and seek any differential effects produced by these mutations; 4) Define the full phenotypic spectrum associated with both ligand and receptor mutations to gain a fuller appreciation of their biology; 5) Contrast the phenotypic spectrum of mutations in the ligand, PROK2, with those in its receptor, PROKR2, to determine whether this system is a single ligand/receptor pathway or not; and 6) Continually refine our understanding of the biology of this new pathway by these detailed human phenotyping studies as well as other findings in the literature that will evolve during this project.

Step 1: Define All PROK2/PROKR2 Mutations in our Population

Given the biological value of our population, the detailed nature of their phenotyping, the novelty of each new mutation/phenotype in these early days of the prokineticins, and the clear difference between some of the nuisances of the mouse vs human phenotypes we have already documented in Prel. Res., it is important to sequence the coding regions of both PROK2 and PROKR2 in the large number of remaining NIH/KS patients (700+) and available family members in our evolving database as well as in our 300 controls with normal reproductive histories and normal physical examinations. ENSEMBL (www.ensembl.org) and UCSC (www.genome.ucsc.edu) genome databases are used to determine the exon-intron structures, design PCR primer sets for the amplification of exons and exon-intron boundaries using PRIMER 3 software (www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and identify the single nucleotide polymorphisms (SNPs) of PROK2 and PROKR2. Any sequence changes encountered are always re-sequenced to confirm each and classify it as a mutation, a rare sequence variation, or a common polymorphism using the evolving HapMap data (www.hapmap.org/cgi-perl/gbrowse/hapmap_B35/) and our control population. Sequence changes resulting in truncated proteins, frameshifts, insertions, or deletions will be categorized as likely mutations. Changes absent from dbSNP and expressed sequence tags and from our 300 ethnically matched healthy controls with normal reproductive functions will be identified as rare sequence variants and possible mutations pending further biological confirmation. Each change will be recorded in our Progeny database curated by Ms. Virginia Hughes, our database coordinator.

Step 2: Biological Confirmation and Characterization of New Mutations

PROKR2 - All confirmed DNA sequence changes in PROKR2 not previously subjected to biological confirmation in our lab or in the literature will then be examined for their in vitro function by Dr. Yisrael Sidis, our molecular biology Co-Investigator. Mutations will be introduced into V5 C-terminally tagged PROKR2 cDNA in pCDNA3.1-V5 expression vector, using the Quick Change XLII Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and will be confirmed by direct sequencing.

Functional analysis - Biological function will be assessed using two different bioassays: 1) a gene transcription assay using the Egr-1-Luciferase reporter containing the murine Egr-1 (Early growth response -1) element promoter region (-1023 to +1) inserted into the pGL3 basic vectors whose activation mainly reflects activation downstream of the ERK signaling cascade (44-46); and 2) a Ca²⁺ influx assay utilizing the photoprotein aequorin that reflects effects of PROK2 signaling on cell function such as motility and secretion in addition to activation of gene transcription. PROKR2 mutations that will have differential activity between these two assays (Fig. 6 Prel. Res.), may reveal functional domains and function/structure relationship within the PROKR2 receptor. The luciferase reporter assay will be performed in HEK293 cells transiently transfected with WT or mutant PROKR2 plasmid together with Egr-1-luc reporter, using Effectene reagent (Qiagen). Transfected cells will then be stimulated with increasing doses of WT PROK2 (10⁻⁶ - 10⁻¹² M range, supplied by our collaborator, Dr. Qun-Yong Zhou cf. below) and luciferase induction will be analysed 48 h post-transfection, using Luciferase Assay System (Promega, Madison, WI). Dose response curves will be generated using the four parameter logistic model (GraphPad Prism 4) and the mutant activity will be determined at the EC₅₀ of the WT response.

Or this?

Specific Aim #2: To characterize the *in vitro* nature of mutations in the *PROK2/PROKR2* pathway found in humans with GnRH deficiency

Hypothesis 3: Mutations in both the ligand and receptor in the prokineticin 2 pathway causing human GnRH deficiency will represent loss of function mutations.

Hypothesis 4: A subset of mutations in *PROKR2* will produce differential defects on downstream signaling pathways that will refine our understanding of the structure-function of *PROKR2* receptor signaling in GnRH neurons.

Overview of our Approach to SAs #1 & 2

Our Preliminary Results provide clear evidence that the spectrum of reproductive disorders associated with mutations in the prokineticin system, even in this small number of early patients, is broad and complex. They occur in both sexes, involve defects at multiple levels of the reproductive axis (i.e., hypothalamic and gonadal), and likely involve important non-reproductive defects such as circadian rhythm abnormalities. Hence, detailed phenotyping and genotyping of patients with mutations in this system will be important. We have clear preliminary data that these DNA sequence changes result in loss of function *in vitro*. Consequently, we will approach **SAs #1 & 2** in a coordinated fashion that involves 6 distinct steps:

- 1) Determine all DNA sequence changes representing putative mutations in *PROK2* and *PROKR2* in our cohort of >900 probands with defects in the GnRH pacemaker and well-phenotyped nlHH/KS/AHH;
- 2) Characterize the *in vitro* biologic activity of each of these changes; classify it as a loss/gain of function; and determine its *in vitro* severity;
- 3) Examine each mutation in at least 2 separate second messenger signaling assays to characterize its impact in each and seek any differential effects produced by these mutations;
- 4) Define the full phenotypic spectrum associated with both ligand and receptor mutations to gain a fuller appreciation of their biology;
- 5) Contrast the phenotypic spectrum of mutations in the ligand, *PROK2*, with those in its receptor, *PROKR2*, to determine whether this system is a single ligand/receptor pathway or not; and
- 6) Continually refine our understanding of the biology of this new pathway by these detailed human phenotyping studies as well as other findings in the literature that will evolve during this project.

Step 1: Define All *PROK2/PROKR2* Mutations in our Population

Given the biological value of our population, the detailed nature of their phenotyping, the novelty of each new mutation/phenotype in these early days of the prokineticins, and the clear difference between some of the nuisances of the mouse vs human phenotypes we have already documented in Prel. Res., it is important to sequence the coding regions of both *PROK2* and *PROKR2* in the large number of remaining nlHH/KS patients (700+) and available family members in our evolving database as well as in our 300 controls with normal reproductive histories and normal physical examinations. ENSEMBL (www.ensembl.org) and UCSC (www.genome.ucsc.edu) genome databases are used to determine the exon-intron structures, design PCR primer sets for the amplification of exons and exon-intron boundaries using PRIMER 3 software (www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and identify the single nucleotide polymorphisms (SNPs) of *PROK2* and *PROKR2*. Any sequence changes encountered are always re-sequenced to confirm each and classify it as a mutation, a rare sequence variation, or a common polymorphism using the evolving HapMap data (www.hapmap.org/cgi-perl/gbrowse/hapmap_B35/) and our control population. Sequence changes resulting in truncated proteins, frameshifts, insertions, or deletions will be categorized as likely mutations. Changes absent from dbSNP and expressed sequence tags and from our 300 ethnically matched healthy controls with normal reproductive functions will be identified as rare sequence variants and possible mutations pending further biological conformation. Each change will be recorded in our Progeny database curated by Ms. Virginia Hughes, our database coordinator.

Step 2: Biological Confirmation and Characterization of New Mutations

PROKR2 - All confirmed DNA sequence changes in *PROKR2* not previously subjected to biological confirmation in our lab or in the literature will then be examined for their *in vitro* function by Dr. Yisrael Sidis, our molecular biology Co-Investigator. Mutations will be introduced into V5 C-terminally tagged *PROKR2* cDNA in pCDNA3.1-V5 expression vector, using the Quick Change XLII Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and will be confirmed by direct sequencing.

Functional analysis - Biological function will be assessed using two different bioassays: 1) a *gene* transcription assay using the Egr-1-Luciferase reporter containing the murine Egr-1 (Early growth response -1) element promoter region (-1023 to +1) inserted into the pGL3 basic vectors whose activation mainly reflects activation downstream of the ERK signaling cascade (44-46); and 2) a Ca²⁺ influx assay utilizing the photoprotein aequorin that reflects effects of *PROK2* signaling on cell function such as motility

What to Look for in a Shorter Application: (think like a reviewer)

New Research Plan Components:

- **Specific Aims**
 - Includes new language about the *impact* of the proposed research
- **Research Strategy**
 - New section includes current Background and Significance, Preliminary Studies/Progress Report, and Research Design and Methods

Facilities and Equipment

- Reflects the Environment criterion
- For ESIs describes the institutional investment in the success of the investigator

Biographical Sketch

- Requires Personal Statement; no more than 15 pubs based on recency, importance to field, and /or relevance to the application



What to Look for in the Revised Criteria: Investigators

- **Personal Statement:**
 - Why their experience and qualifications make them particularly well-suited for their roles in the project
- **Publications:**
 - Recommended: no more than 15---up to five of the *best*; up to five of the *most relevant* to the proposed research; up to five of the *most recent*
 - If *Early Stage Investigators or New Investigators*, do they have appropriate experience and training?
 - If *Established*, have they demonstrated ongoing record of accomplishments that have advanced their field(s)?



What to Look for in the Revised Criteria: Innovation

- Does application challenge/seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions?
- Concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense?
- Refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?
- ***Not all applications need to be innovative !***



What to Look for in the Revised Criteria: Approach

- **Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project?**
- **Are potential problems, alternative strategies, and benchmarks for success presented?**
- **If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed?**



What to Look for in the Revised Criteria: Approach

If the Project Involves Clinical Research:

Are plans justified for:

- **protection of human subjects**
- **inclusion of minorities, both sexes/genders, and children**



What is the Difference Between Impact and Significance ?

Impact addresses:

- Probability of whether the research will exert a sustained, powerful influence on the research field.

Significance addresses:

- Does the project address an important problem or a critical barrier to progress in the field?
- If the aims are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved?



What to Look for in the New Facilities and Equipment Section?

- Limited to those resources directly applicable to the proposed work:
 - ESIs describe institutional investment, e.g., start-up funds and mentoring arrangements.
 - For multiple sites, resources at each site should be described.
 - Special facilities that handle biohazards, etc., included.
 - Major items of equipment already available for the proposed studies listed under Equipment.



Links of Interest

Enhancing Peer Review: The NIH Announces Enhanced Review Criteria for Evaluation of Research Applications Received for Potential FY2010 Funding

<http://grants.nih.gov/grants/guide/notice-files/not-od-09-025.html>

PageLimits: http://enhancing-peer-review.nih.gov/page_limits.html

Human subjects: <http://grants.nih.gov/grants/policy/hs/index.htm>

Vertebrate Animals: <http://grants.nih.gov/grants/olaw/olaw.htm>

SF424 guidelines for submission:

<http://grants.nih.gov/grants/funding/424/index.htm>