Grants 101 Part II: Training and Career Development Awards

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DOM Fellows Course –Surviving and Thriving in the Research Years

August 30, 2021

Thanks to Sheila Lukehart, PhD for sharing her slides and wisdom on this topic.

Grants 101

I. Grants 101 Part I: Introduction to Research Administration

Monica Fawthrop

II. Training & Career Development Awards

Ellen Schur

III. NIH Structure & Behind the Scenes at Study Section

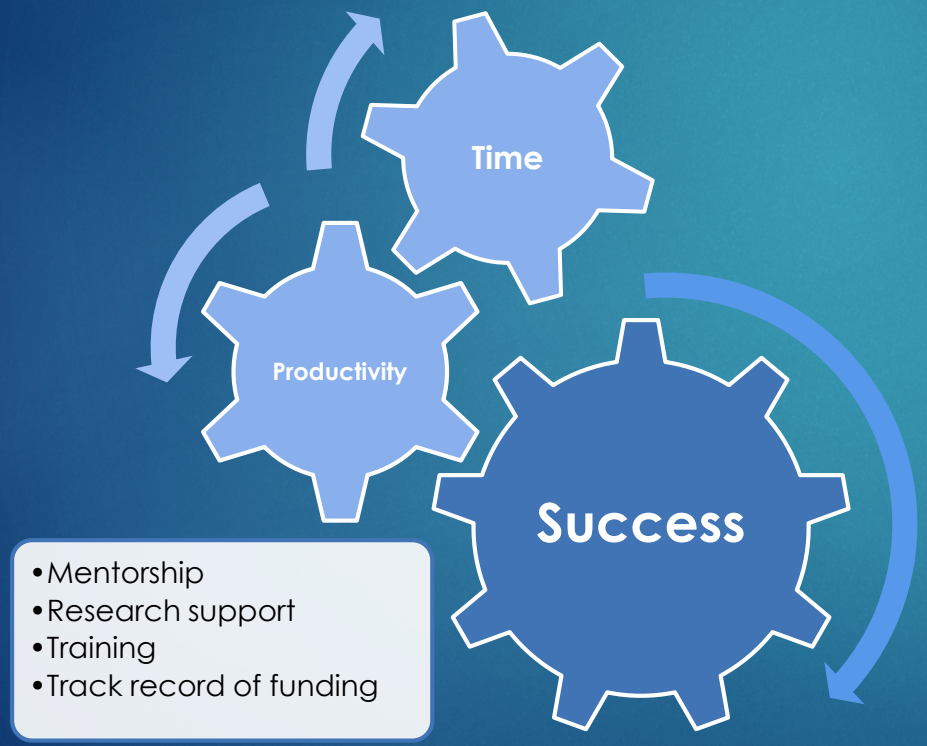
Tom Hawn

Training and Career Development Awards

- Purpose of a career development award
- Selecting a career development award
  - Where to get information
- Components of an NIH K application
- Tips on writing a great application

Purpose of a Career Development Award

For you



For sponsor/NIH

- Develop and shape the scientific workforce
- For foundations and NIH Institutes, promote scientists devoted to their area of emphasis
- Target support to specific stages of career development
- Target support to improve representation in the scientific workforce

Example of a Career Development Award (K award)

- Mentored Career Development Award
  - Early career
  - Postdoc or junior faculty
  - Examples: K08, K23, K01
- Require 75% protected time for research and training
- 3-5 years duration
- Salary support ($50-100K per year)
- Modest funds for research
- Mentor(s) required (but not paid by the grant)

Tips on writing a great application

This grant is about you

Selecting a career development award

Eligibility

- MD or clinical degree vs. PhD
- Years since doctorate
- Person from underrepresented background
- Citizenship

Research

- Patient-oriented or basic
- Clinical trial or not
- Specialized – e.g., engineering

Institute

- Topic of your research
- Mechanisms offered
- Funding levels

Selecting a career development award: Eligibility

- US citizen, permanent resident
  - K08
  - K23

  } MD, DVM, DDS, other Clinical Doctorate

  - K01
  - K22
  - K25

  } MD or PhD

- US Citizen/PR or Non-citizen
  - K99/R00 Pathway to Independence: MD or PhD
  - Many foundations do not have citizenship requirements

Selecting a career development award: Research

K08: Mentored Clinical Scientist Research Career Development Award

- Laboratory focused research
- May use human samples

K23: Mentored Patient-Oriented Research Career Development Award

- Patient oriented research
  - Clinical trial not allowed
  - Clinical trial required
  - Independent basic experimental studies with humans required

Eligibility: MD, DVM, DDS, other Clinical Doctorate

Selecting a career development award: Research

K01: Mentored Research Scientist Development Award

- Basic or clinical
- Institute-specific purposes

K25: Mentored Quantitative Research Career Development Award

- Quantitative science or engineering degree moving to health-related topics/biomedical research

Eligibility: PhD or MD, DVM, DDS, other Clinical Doctorate

Selecting a career development award: Institute

- Some institutes don't offer all grant mechanisms
- Some institutes have special mechanisms or requirements
  - NIAID: K99/R00 Physician/Scientist MD only
  - NHLBI: K01 Mentored Career Development Award to Promote Faculty Diversity in Biomedical Research
- Pay lines vary
  - Optimize your chance of success if possible

Selecting a career development award: Transition to independence awards

K99/R00 NIH Pathway to Independence Award

- Postdoc ***move*** to Assistant Professor
- No more than 4 years of postdoctoral research* experience at the time of submission (or resubmission)
- Has mentored postdoc phase K99 (1-2 years)
- Independent Asst. Prof. phase R00 (up to 3 years)
- ***Non-citizens eligible***

K22 Career Transition Award

- Mentored phase (postdocs eligible)
- Followed by independent research phase
- NIAID, NCI only

Tips on writing a great application

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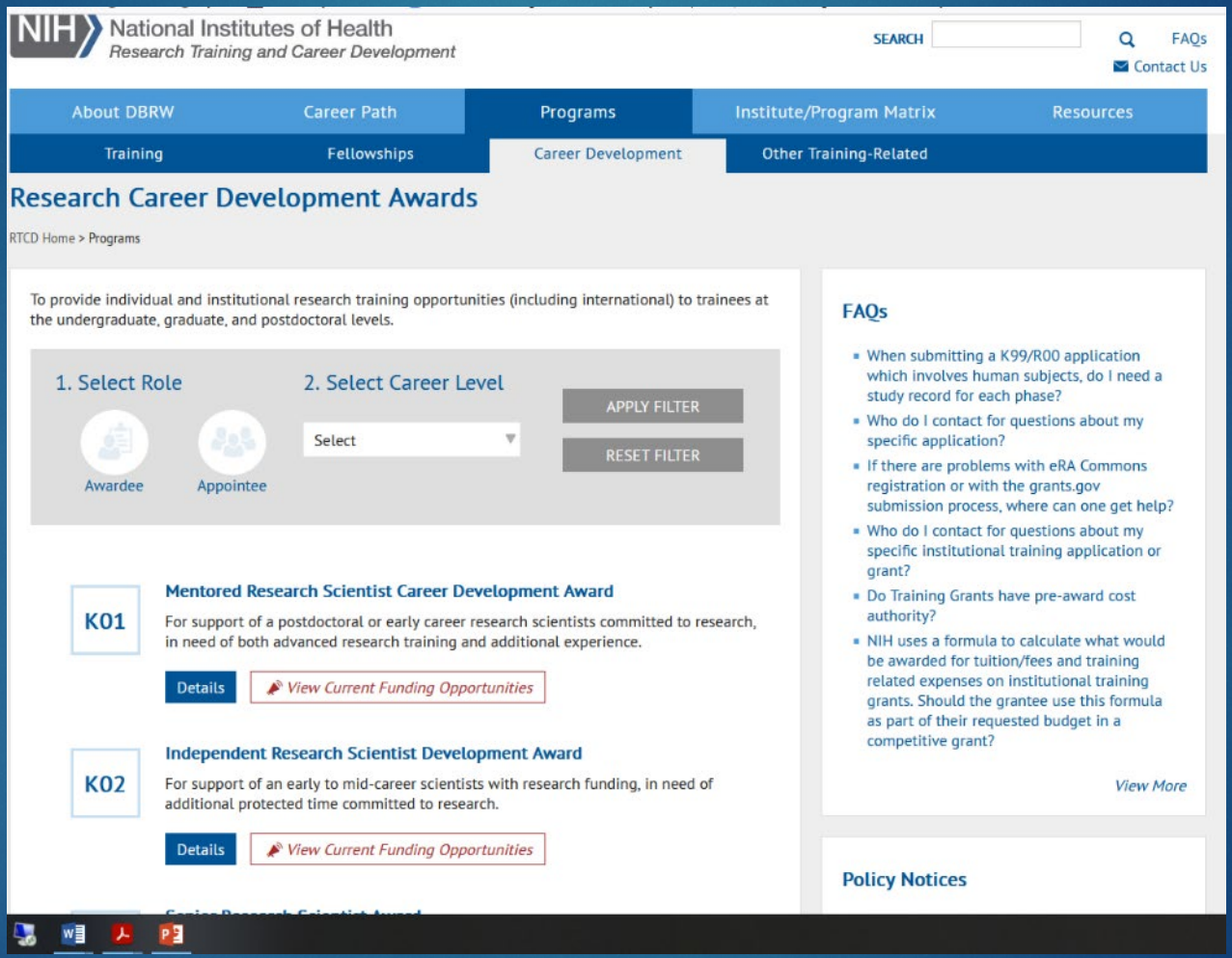
Where to get more information



https://researchtraining.nih.gov/programs/career-development

Training and Career Development Awards

- Purpose of a career development award
- Selecting a career development award
  - Where to get information
- Components of an NIH K application
- Tips on writing a great application

This is the most important slide of my talk

- How you present your science matters, too
- Use formal language—no slang or jargon
- Use correct grammar and punctuation
- No typos!
- Pay attention to required fonts, margins, page limits

PUT YOUR BEST FOOT FORWARD

Tips on writing a great application

This grant is about you

Match you, your research, and your mentor to the mechanism and institute

Inspire confidence in your potential with a well-presented proposal

Boring—and causes tired eyes……

these two lipoproteins as adhesins. The strain expressing both DbpA and DbpB acquired the ability to bind epithelial cells while only DbpB showed specificity for glioma cells *in vitro* (5). Later studies with the neuroborreliosis patients validated our results since antibodies mainly against DbpB were present in CSF after colonization by Lyme spirochetes (4, 12). Therefore, we anticipate that our *in vitro* experiments in the initial screen using non-infectious *B. burgdorferi* will identify surface localized *T. pallidum* adhesins. This non-adherent strain offers a cleaner background to study binding mechanisms since it does not express *B. burgdorferi* adhesins. Candidate adhesins identified from this experiment will help us select 3-4 surface proteins to express in the infectious, bioluminescent *B. burgdorferi* strain. ¶

We will first select the best luciferase reporter system and most useful promoter to express this reporter for *in vivo* imaging in the small animal model. Then, we will express and characterize the promising *T. pallidum* proteins, identified from the initial screen, in the infectious, sequenced *B. burgdorferi* strain to assess adherence to placental and neuronal cell lines *in vitro*. These results will form a foundation for our *in vivo* assessment of *T. pallidum* proteins in colonization of placenta and neuronal tissues. Hence, using the gain of function approach *in vitro* will allow us to test its validity also in the mouse model of infection. ¶

1A. Identification and characterization of *T. pallidum* adhesins with affinity for placental and/or neuronal tissues and other virulence factors. We have selected several genes of *T. pallidum* for the initial screen to determine them as candidate adhesins in this study. We will obtain clones containing these genes from Drs. Sheila Lukehart and Arturo Centurion at University of Washington at Seattle (please see their letters of support). We will also produce respective recombinant tagged proteins in *E. coli* and generate polyclonal antibodies against the proteins for which antisera are not available from our collaborators. ¶

We considered different features in selection of these proteins, such as; they (i) are known to be expressed during congenital syphilis or neurosyphilis on the basis of serological analysis, (ii) show specificity to a particular host receptor expressed in placenta and/or neuronal tissues, (iii) exhibit other potential activities important for pathogenesis, and (iv) were previously described membrane proteins with unknown function. Selected eight *T. pallidum* proteins, TP0171, TP0319, TP0435, TP0574, TP0954, TP0957, TP0971, and TP1037 have potential to contribute to neurosyphilis or congenital syphilitic manifestation. We will clone the genes along with their promoters in *B. burgdorferi* shuttle vector and transform the non-infectious *B. burgdorferi* B314 strain, which was also used to examine role of DbpA-DbpB, as described above (rationale). We will first assess the function of *T. pallidum* proteins expressed in *B. burgdorferi* as a surrogate system *in vitro*. Expression of *T. pallidum* genes in *B. burgdorferi* will be confirmed by Western blotting. Some of the selection criteria for candidate proteins are described here. ¶

(i) Several immunogenic proteins are identified but their functions not yet determined. TP0171 is a 15kD lipoprotein, which shows homology to proteins of *Listeria monocytogenes* and *L. innocua*, two pathogens causing adverse outcomes in pregnant women. TP0171 is a major membrane immunogen in *T. pallidum*. TP0435 (17kD) lipoprotein and TP0574 (previously known as TpN47) are two highly immunogenic proteins used in diagnosis of syphilis. However, their localization on the spirochete surface remains questionable and their roles have not been examined. This study will unequivocally determine their subcellular localization in the spirochete and will help us evaluate their roles. If one or more of these proteins are present on the spirochete's surface in our initial screen, they will be selected for further experiments. ¶

(ii) Based upon a comprehensive analysis of the available information, we anticipate that TP0954 protein may located on the outer membrane and may facilitate colonization of placenta and neuronal tissues by *T. pallidum*. If so proved, it will provide a model molecule to study molecular basis of congenital spirochete transmission and neurosyphilis. We anticipate that TP0954 encoded protein will be located on the surface of the *T. pallidum* since it possesses a potential signal peptide. In addition, the predicted 3D-structure of this protein using the Hiden Marko models (HMM) program with Protein Data Bank (PDB) shows similarity with several surface proteins in other organisms. These similar proteins include the PilF outer membrane lipoprotein of *Pseudomonas aeruginosa*, peroxisomal-targeting signal 1 binding domain of *Trypanosoma brucei* Peroxin 5 protein, and yeast mitochondrial outer membrane translocon protein Tom70p. All possess tetratricopeptide repeats. Finally, one peptide of TP0954 showed 54% similarity with defined chondroitin sulfate A-binding variable domain of PfEMP1 *Plasmodium falciparum*. Furthermore, PfEMP1 of malaria parasite displayed on infected red blood cells (RBCs) promotes adherence of the RBC to placenta. Interestingly, we have previously shown that DbpB lipoprotein of *B. burgdorferi* shows affinity to chondroitin sulfates and mediates binding to the glial cells. Later analyses of cerebrospinal fluid from neuroborreliosis patients confirmed intrathecal (in situ) expression of DbpB by Lyme spirochetes (4, 12). This collective information strongly supports inclusion of this protein in this proposal. ¶

(iii) TP1037 encoded protein is designated as hemolysin III in the genome. Any organ can be affected due to *T. pallidum* dissemination after infection of the fetus by this spirochete. Anemia is common in congenital syphilis and non-hemolytic anemia can persist for weeks even after treatment (21). It will be useful to determine if hemolysin III of *T. pallidum* is involved in this manifestation. Hemolysis on blood agar plates stimulated by *T. pallidum* hemolysin III will determine its enzymatic activity *in vitro*. These experiments will functionally establish its current predicted role on the basis of sequence homology with proteins of other pathogens. In addition, we will determine in our later experiments whether the expression of this hemolysin results in anemia in mice, similar to that seen in some syphilis patients and in congenital syphilis. ¶

(iv) We have selected three more proteins, which are known membrane proteins with unknown functions. First, Treponema-specific membrane lipoprotein (*tmpC* or TP0319) is an ABC-type nucleoside transport system that may transport purine nucleosides, which are essential for the survival of *T. pallidum* within its obligate human host. If it is not exposed to the surface of the spirochete in the initial analysis, it will serve as a negative control for all following experiments in the specific aim 2. Second, Dr. Norgard's group recently crystallized the membrane antigen (*tpd* or TP0971) of *T. pallidum*. It shows high affinity for human lactoferrin, suggesting its role as iron scavenger. These two proteins, TmpC and Tpd, are expressed at high levels in *T. pallidum* during infection (19) but their contribution to *T. pallidum* pathogenesis remains to be established. The current study will determine if they are located on the surface and potentially play a role in survival of the spirochetes in specific tissues during infection. Third, TP0957 encoded protein belongs to the extracellular solute-binding transporter superfamily that also includes sialic acid-binding protein in other bacteria. Sialic acids are found widely distributed in mammalian tissues. They are also components of gangliosides and are found attached to the glycosphingolipid (ceramide and oligosaccharide). Since gangliosides are predominantly found in the nervous system, TP0957 could be a potential adhesin for neuronal tissues. ¶

Although some of these selected proteins were initially predicted to be periplasmic proteins, Hazlett and coworkers (2005) showed that several periplasmic proteins of *T. pallidum* can get exposed due to outer membrane destablization facilitated by outer membrane protein encoded by TP0453 (7). Therefore, it is useful to determine exact location of these proteins and assess their roles in colonization of neuronal and/or placental tissues. ¶

1B. Evaluation of *T. pallidum* proteins in adherence to cell lines derived from human placenta and neuronal tissue. Colonization of specific tissues *in vivo* often can be predicted on the basis of *in vitro* binding experiments conducted with relevant cell lines and the pathogen. The focus of this study is to identify proteins important in colonization of placental and/or neuronal tissues. Therefore, we will use the human epithelial cell line obtained from placental choriocarcinoma, CCL-98, and fibroblast cell line, CRL7464 as model for placental colonization, while neuronal cell line, PC12, and C6 glioma cell lines will be used to depict colonization of the central nervous system (CNS) during infection. Radiolabeled *B. burgdorferi* will be used in the binding experiments to assess the contribution of *T. pallidum* proteins in adherence with the gain of function approach. The wells without the cell line monolayers, and *B. burgdorferi* strain transformed with the shuttle vector alone will provide negative controls for specific mammalian cells and expressed *T. pallidum* protein, respectively. A significantly higher level of adherence by *B. burgdorferi* expressing specific *T. pallidum* protein(s) on their surface to these cell lines, as compared to *B. burgdorferi* control will identify them as adhesin(s). In addition, these results will suggest potential role of these proteins in colonization of specific tissues by *T. pallidum* during infection of humans. We have extensive experience in conducting these experiments with *B. burgdorferi* and found them to be very useful in identifying the bacterial adhesins and host receptors, and predicting their contribution in specific tissue colonization *in vivo*. ¶

Visual Appeal

- Open space
- Clear organization
- Use of Bold, CAPITALS, underlining, and outlining to define sections
- Theoretical models, diagrams, and figures
- Color or grayscale
- Not too small

EXPECTED RESULTS AND INTERPRETATION Based upon our experience with TprK,[62] we expect that antibody specificity will be detected among different sequences for a given DR, and that the number of AA changes necessary to abrogate antibody binding will be few. We expect that antibodies will bind to sequences in the predicted loops, but these loops also contain conserved sequence in addition to the DR, so we cannot predict now whether there will be cross-reactive antibodies that bind the conserved regions of these loops. If so, this may have implications for the specificity of opsonization and neutralization, and may argue against a major role for TprC and D subspecies- and strain-specific immunity. The role of the conserved regions (within loops and separate from loops) in functional immunity, including cross-protection, will be explored formally using a complementary approach in Aim 4. Those results, along with results from Aims 2 and 3, will be evaluated together to reach conclusions or to develop further hypotheses.

LIMITATIONS AND ALTERNATIVE APPROACHES Completion of Aim 2 will require successful production and purification of a large number of recombinant proteins and peptides. OM proteins can be quite difficult to express in *E. coli*. We have been expressing Tpr proteins and other putative OM proteins from *T. pallidum* for ~15 years. The laboratory has used a number of different vectors, host strains, and growing conditions in order to optimize expression for individual molecules. We routinely express such proteins without the signal sequence to avoid toxicity to *E. coli*. Even so, the protein is often found in inclusions, which requires solubilization in urea or other agents before it can be purified (we typically use 6XHIS-tags for purification). Depending upon its intended use, the quality of the antibody that is produced following immunization with recombinant proteins is dependent upon the correct folding of the immunizing protein: if one wants an antibody simply to identify a protein in an immunoblot, correct folding is not necessary; if one wants antibody to recognize a 3-dimensional structure on an intact bacterium, however, correct folding may be critical. Lack of appropriate attention to this issue may be the reason that functional assay results obtained in one laboratory may not be successfully reproduced in another lab. For the proteins that are produced in this project, conditions for optimal folding will be determined, and the degree of correct folding will be evaluated by circular dichroism. Figure 5 shows an example of purified recombinant TprK (predicted to have a structure very similar to TprC and D) that has been optimally refolded in our lab; the spectrum is typical of a molecule rich in β-sheets, consistent with β-barrel structure. Purity of our recombinant proteins and peptides will be assessed by SDS-PAGE and immunoblotting (using anti-6xHIS and infection-immune rabbit serum). If further purification is needed, size exclusion chromatography will be used. Synthetic linear and cyclic peptides will be obtained commercially. We have considerable experience with performing ELISA and lymphocyte proliferation assays using whole recombinant proteins and synthetic peptides as antigens; we don't anticipate any problems with these assays.[56,57,59-61]

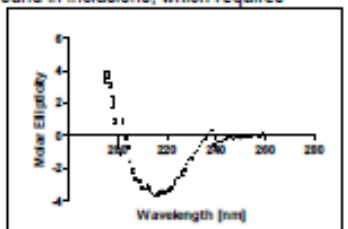


Figure 5. CD spectrum for purified refolded recombinant TprK, another likely OM protein of *T. pallidum*. The spectrum indicates abundant β-sheet composition.

Aim 3. Determine the role of the distinct regions of TprC and D in functional immunity, using homologous and heterologous *T. pallidum* strains as the targets of the functional assays.

RATIONALE AND PRELIMINARY DATA
Antibody can facilitate the killing of *T. pallidum* in two ways: opsonization for phagocytosis by macrophages,[63] and complement-mediated neutralization.[64] It is now widely believed that the major mechanism of clearance of *T. pallidum* from early lesions is by opsonophagocytosis, so the identification of the targets of opsonic antibody has been long-sought. Such targets are also surface-exposed antigens, so opsonization of *T. pallidum* has been used as a functional assay for surface-exposure of an antigen of interest. Several proteins have been reported to be opsonic targets in *T. pallidum*, including TprK,[44] although acceptance of these results has not been universal.[65] Data presented above indicate that several of the Tpr proteins, including TprC and TprD are also targets of opsonic antibody, and 3D

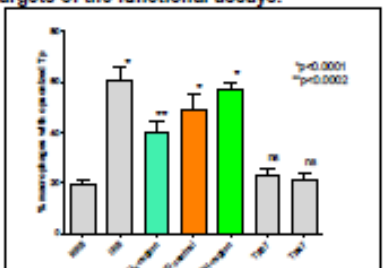
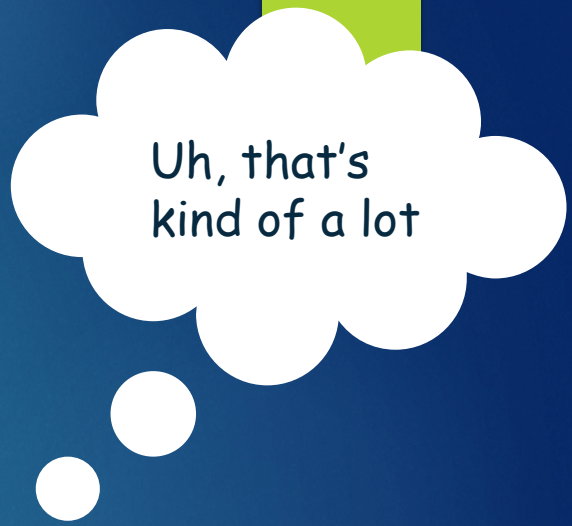


Fig. 6. Opsonization of *T. pallidum* Nichols strain by antisera directed to recombinant peptides of TprC/D.

Components of K Applications



Major Sections

- Specific Aims
- Candidate Section
- Mentor's statement, Co-Mentors
- Environment
- Institutional Commitment to Candidate
- Research Plan

Minor Sections

- Project Summary Abstract
- Project Narrative
- Human Subjects
- Vertebrate Animals
- Training in Responsible Conduct of Research (1 page)
- Authentication of Reagents
- Biohazards
- Select Agents
- Letters of Support (Collaborators)
- Budget
- Budget Justification
- Resource Sharing Plan
- Biosketches for You, Mentor, Co-mentors
- Letters of Reference (3-5 letters from people who know you)

Components of K application

Section	Pp	Purpose	Time	Importance
Specific Aim	1	What are they funding?	↑↑↑↑↑	Inspires; good first impression
Candidate	~4*	Who are they funding?	↑↑↑	Explains; logical extension of prior training; learning needs
Mentor's statement	6	Who will be helping you?	↑ Mentor	Reassures; weaknesses will be overcome
Environment	1	Do you have resources?	↑	Expected; checks a box
Institutional commitment	1	Do you have support to become independent?	↑ Dept.	Fundamental; establishes basis of academic career
Research Plan	~8*	What science will you do… in the current research? in the future?	↑↑↑↑↑	Engages; interesting, rigorous science with potential for growth

* 12 pp limit combined

Specific Aims

- One page summary of the application
- An opportunity to tie the research proposed to your training plan and career trajectory
- Introduce your research plan
  - What is the hypothesis(es), and what data/literature support it?
  - What are the exciting new preliminary data that support your aims? Which data are YOURS?
  - What are you going to do?
  - What will your results mean for the field?
  - What will this project mean for your career?

What are they funding? (and why they should absolutely, positively do so!)

Tips on writing a great application

This grant is about you

Match you, your research, and your mentor to the mechanism and institute

The Specific Aims page is the most critical page in the application

Inspire confidence in your potential with a well-presented proposal

Candidate Section

- Candidate's Background
  - How did you get where you are?
  - Let the reviewers get to know you
  - Hint: establish your track record and commitment to a career in science
- Career Goals and Objectives
  - Where do you see yourself in 5 or 10 years?
  - Hint: "…independent investigator in the field of _____"

Who are they funding? (and why it makes sense to fund you to do this work)

Candidate Section

- Career Development/Training Activities
  - How will this award fill your training gaps?
  - How will this training be foundational for your anticipated future research?
  - What to include:
    - Didactic coursework
    - Technical training
    - Skills enhancement (e.g., grant writing)
    - Local and national conferences

Tips on writing a great application

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It's OK to have weaknesses; address them in training section

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Mentor Statements (6 pages total)

- Mentor's statement should include
  - Evidence of successful training history
  - Evidence of active productive research
  - Evidence of support for proposed research
  - Details about mentoring—e.g. frequency of meetings
  - Topic areas in which mentoring will occur
  - Plan for transitioning candidate to independence
- Co-Mentors' statements should be specific about the expertise that they bring to the mentoring team

Who is helping you? (and why they are qualified to do so)

Should match your Candidate section

Environment & Institutional Commitment to the Candidate

- Description of Institutional Environment (1 page)
  - Intellectual environment
  - Facilities, resources
- Institutional Commitment to Candidate's Research Career Development (1 page)
  - Is usually letter from Chair/Division Head
  - Guarantees >75% protected time for research training
  - Lab space, office, academic appointment

Do you have resources and support to become an independent investigator? (the answer should always be Yes)

The Science: Last, But Certainly Not Least!

- Schedule uninterrupted time to sit and think—days of time
- Read the latest papers in your field and well-written review articles
- What are the unknowns in the topic that you are studying?
- How do the ideas in your proposed research connect…
  - to each other?
  - to existing literature?
  - to your career goals?
- *Follow your heart as well as your mind*

Research Plan

- Specific Aims—1 page (not in 12-page limit)
- Research Strategy
  - Significance
  - Innovation
  - Approach

What science will you do? (and how it will help you in your career)

Significance (Background)

- Explain the importance of the problem
  - Clinical conundrum, scientific question, technical barrier
  - Hint: what excites you about this work?
- Assume you are not writing for an expert
- Identify gaps in knowledge; state how you will fill those gaps; tie to each Specific Aim
- Avoid selective citation of the literature
  - Hint: areas of controversy need more research!

Innovation

- What is new?
  - Hypotheses and ideas
  - Methods
  - Population
  - Technologies
  - Combining any of above: e.g., applying established methods in new population
- Keep it short and sweet!

Approach: Research Design and Methods

- Organize by Specific Aim*
  - Rationale and Hypothesis
  - Experimental Approach*
  - Expected Results & Interpretation
    - Statistical analysis, sample size
  - Potential Pitfalls and Alternative Approaches
- Other Important Sections
  - Future Directions – R01
  - Timeline – include grants

* For clinical studies, experimental approach might be the same for multiple aims; Organize by Aim for Expected Results and below

Research Plan

SIGNIFICANCE

APPROACH

Ko8 review criteria for Research Plan

- Are the proposed research questions, design, and methodology of significant scientific and technical merit?
- Is the prior research that serves as the key support for the proposed project rigorous?
- Has the candidate included plans to address weaknesses in the rigor of prior research that serves as the key support for the proposed project?
- Has the candidate presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed?
- Has the candidate presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?
- Is the research plan relevant to the candidate's research career objectives?
- Is the research plan appropriate to the candidate's stage of research development and as a vehicle for developing the research skills described in the career development plan?

Tips on writing a great application

This grant is about you

Your research plan doesn't have to change the world, but it should change you career

Match you, your research, and your mentor to the mechanism and institute

Training program must be substantive

The Specific Aims page is the most critical page in the application

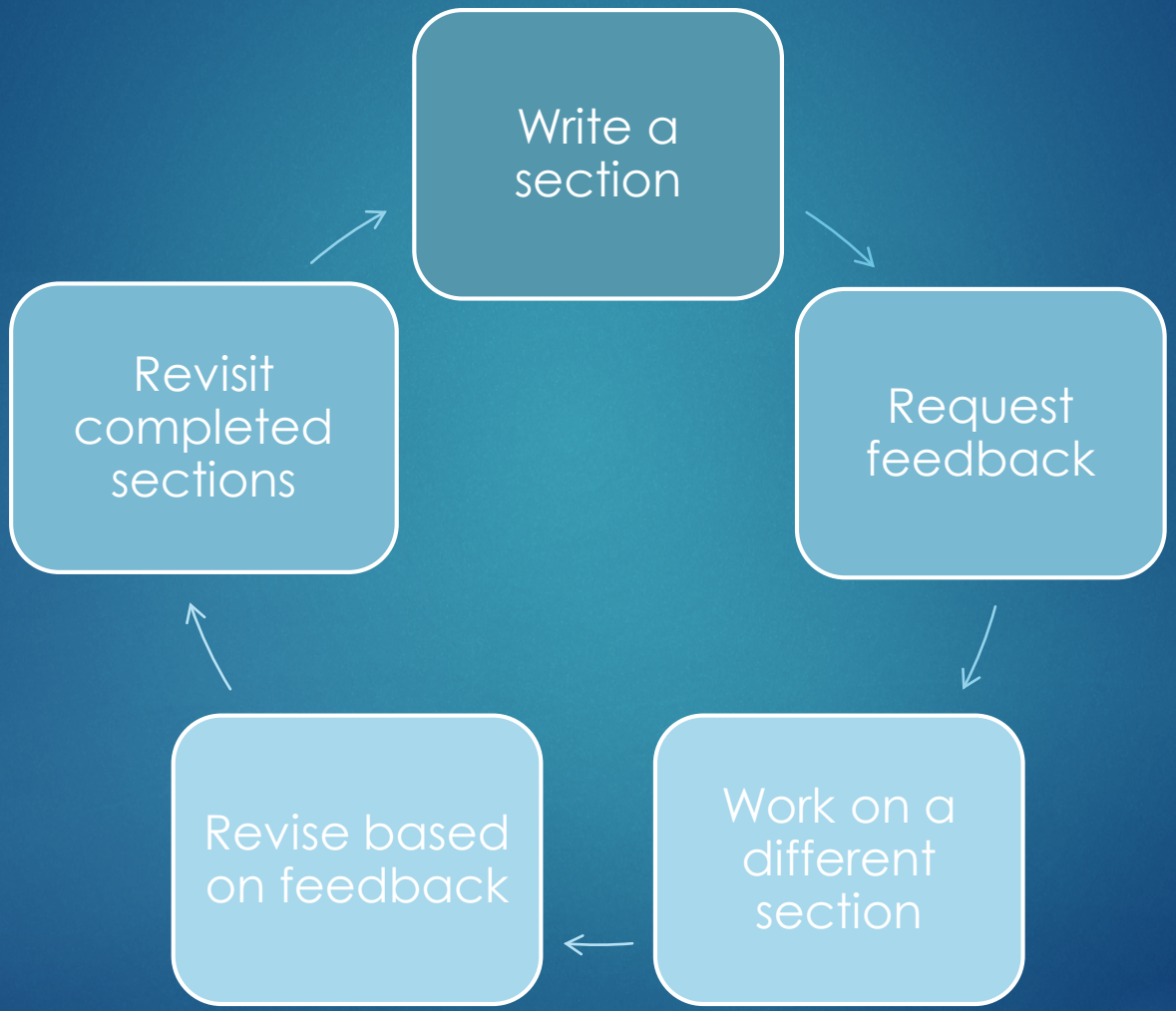
Inspire confidence in your potential with a well-presented proposal

Components of K application

Section	Pp	Purpose	Time	Importance
Specific Aim	1	What are they funding?	↑↑↑↑↑	Isn't this cool?
Candidate	~4*	Who are they funding?	↑↑↑	Aren't I awesome?
Mentor's statement	6	Who will be helping you?	↑ Mentor	We got this!
Environment	1	Do you have resources?	↑	Can do!
Institutional commitment	1	Do you have support to become independent?	↑ Dept.	We are all in.
Research Plan	~8*	What science will you do… in the current research? in the future?	↑↑↑↑↑	My science is real.

* 12 pp limit combined

The iterative writing process



The Rewards!

- Start a rewarding career in science
- Discovery!
- Make a difference!
  - Help to understand, control, prevent, or cure a disease
  - Teach, develop, and train the next generation of outstanding scientists

http://www.nesc.nhs.uk/images/biomedical%20scientists.jpg

Science is real!!

"A scientific theory isn't just a hunch or guess. It's more like a question that's been put through a lot of tests."

- They Might Be Giants, et al. Science is real. *Here Comes Science!* 2009: Track 1.