Sample Template

Specific Aims: NIH

Title

The title should create a good first impression, inform the reviewer of the proposed research topic, and engage the reviewer's interest.

Note: *NIH limits titles to 81 characters (including spaces).*

> **Special Note**
>
> *Ideally, the Specific Aims section of an NIH application should be limited to approximately 1 to 1.5 pages.*
>
> *Also note that NIH now requires that applications be prepared using one of four fonts: Arial, Helvetica, Georgia, or Palatino Linotype.*

1st Paragraph

- Introduce the project.
  - Relate the project to the agency's mission.
- Educate the reviewer.
  - Summarize the important knowledge.
- Identify the gap in the knowledge or state the critical need.
- Identify the problem created by the gap or need.

2nd Paragraph

- Describe your long-range research or career goal(s).
  - Ensure that your long-range career goal aligns with the agency's mission.
- State your overall project goal.
  - Ensure that the overall project goal addresses an identified gap in knowledge and represents a step toward achieving your long-range career goal.
- Present your central hypothesis (or, alternatively, a statement of need).
  - Be sure that you present a true hypothesis – one that can be objectively tested to determine its validity – rather than a predetermined conclusion.
- Explain your rationale for pursuing the project.
  - Indicate what it will be possible to accomplish when your research is complete.
- Describe your qualifications and research environment.
  - How you are better prepared than other, equally qualified researchers.
  - Identify special training, expertise, experience, and, most importantly, relevant preliminary data.
  - Identify access to human and animal subject pools; to unique equipment and instrumentation; and to collaborations and partnerships.

3rd Paragraph

- Delineate your specific aims in a bulleted list.
  - Ensure that specific aims correlate with your central hypothesis.
  - Ensure that all specific aims relate to and support your overall project goal.
  - Provide conceptual rather than descriptive specific aims.
  - Delineate a reasonable number of specific aims, presented in a logical order.
  - "Why" aims are generally stronger than "what" aims.
  - Define a clear purpose, working hypothesis or statement of need, and expected outcome for each specific aim.
  - Make sure no specific aim is dependent on the successful outcome of another aim.

4th Paragraph

- Identify the project's innovation, e.g., a unique approach or technology.
- Delineate the project's expected outcomes.
  - Should validate central hypothesis and resolve gap in knowledge.
- Summarize the project's significance
  - Provides segue to Background and Significance

How to Write A Competitive Grant Proposal: Specific Aims

Erica Whitney

Research and Career Development Manager, QB3

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California Institute for Quantitative Biosciences

Timeline for Proposal Preparation

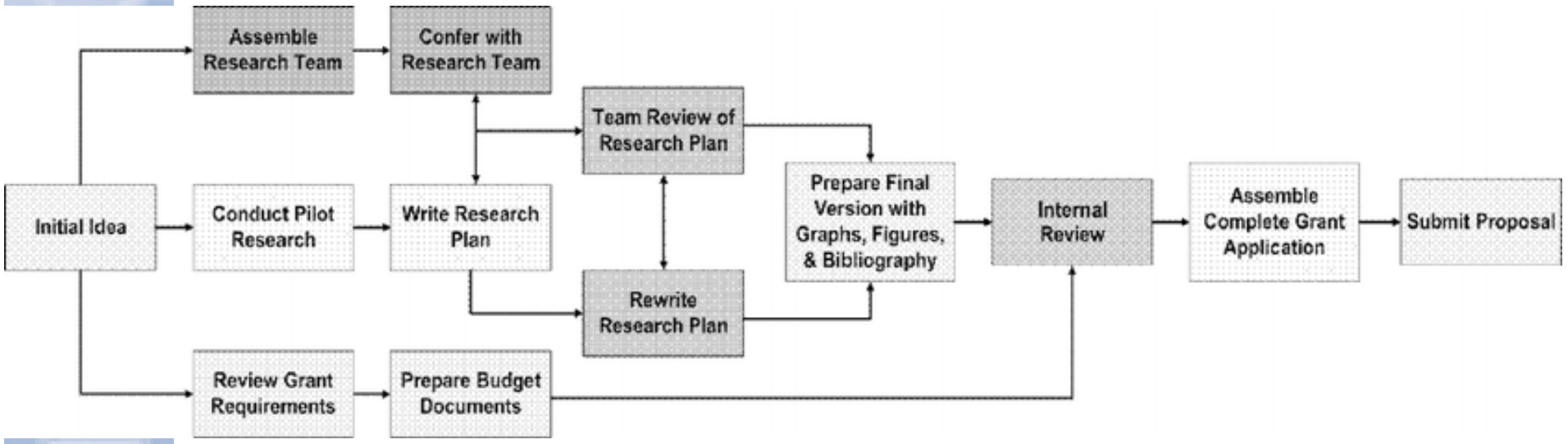

Figure 1 Steps of the NIH grant application process. Steps in preparing and submitting a grant proposal.

Berg, K.M, et. al. J Gen Int Med 2007;22:1587-95

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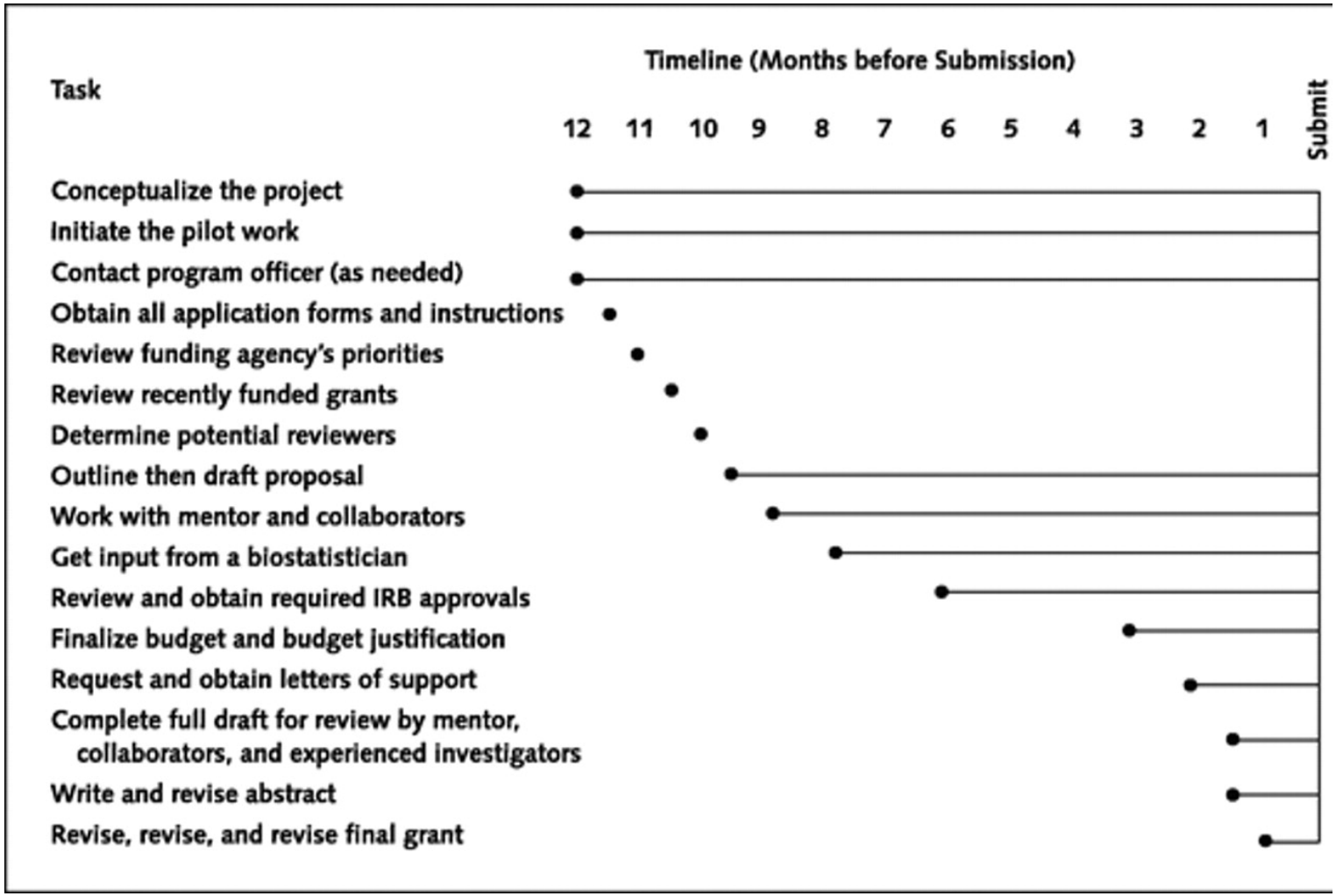
Timeline for Proposal Preparation



Inouye, S. K. et. al. Ann Intern Med 2005;142:274-282

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Most common reasons for not receiving funds*:

- Lack of new or original ideas
- Diffuse, superficial or unfocused research plan
- Lack of knowledge of published relevant work
- Lack of experience in the essential methodology
- Uncertainty concerning the future directions
- Questionable reasoning in experimental approach
- Absence of acceptable scientific rationale
- Unrealistically large amount of work
- Lack of sufficient experimental detail
- Uncritical approach

*Per Cheryl Anne Boyce, Ph.D. and Houston Baker, Ph.D., Grant Writing for Success, NIH Regional Grant Workshop

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What are Your Goals?

- Specific
- Measurable
- Realistic

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Specific Aims

Your Specific Aims are the *cornerstone* of your entire proposal.

- The objectives of your research project.
- What you want to accomplish.
- Your project milestones.
- Testable concepts and ideas.
- Focused on an unresolved issue or on a roadblock to advancing the field.

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Specific Aims

Your specific aims are:

- The most important page of your proposal.
- Start with a brief problem statement to introduce your research question and state why it is important.
- Limited to 2-4 aims.
- Declarative (use short bullet points).
- Explain why this research matters.
- Not inter-dependent but supportive of each other.

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Specific Aims

Include, on 1 page:

Introduction:

-Big picture/relevance of your research.

-The problem you are addressing.

- Focus of project/what you hope to accomplish.

- Overall hypothesis.

- Lead into Specific Aims.

Specific Aims:

-Propose to test mechanistic hypotheses.

-State what you propose to determine or test.

- Specific Aims are not methods; include brief description of approach *after* the Specific Aim.

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2. SPECIFIC AIMS

Insert preamble that describes the unmet medical need and/or gaps in our biomedical knowledge and why this is an important topic of study.

Our long-term goal is to understand _____. The specific objective of this proposal is to _____. The central hypothesis is that _____. We formulated this hypothesis, in part, based upon our strong preliminary data, which shows that _____. The rationale for the proposed research is that once it is known how _____. We will pursue these studies in three Specific Aims:

Aim 1 *INSERT TEXT.*
Our *working hypothesis* for this Aim is that _____.

Aim 2 *INSERT TEXT.*
We will test the *hypothesis* _____.

Aim 3 *INSERT TEXT.*
In these studies, we will examine the *prediction* that _____.

The proposed work is innovative because it capitalizes on _____. At the completion of this project, we expect that the combined work proposed in Aims 1 and 2 will _____. We also expect that Aim 3 will establish _____.

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Introduction

Dr. James R. Alfano, University of Nebraska at Lincoln

Application 1-R01-AI069146-01A2: Suppression of innate immunity by an ADP-ribosyltransferase type III effector

Big picture/ Rele-vance

Background

Problem

Eukaryotic innate immune systems act as effective barriers to infection by microorganisms. Understanding the mechanisms that bacterial pathogens employ to circumvent innate immune systems will improve our ability to control disease. Plants and animals use specific pattern recognition receptors (PRRs) to recognize conserved molecules of microorganisms (known as PAMPs). Plants have numerous PRRs that can recognize specific virulence proteins specifically present in pathogens (known as Avr proteins). Many Gram-negative bacteria use type III protein secretion systems to inject effector proteins into host eukaryotic cells. We have shown that a primary role for many *Pseudomonas syringae* type III effectors is to suppress innate immunity. However, the enzymatic activities and the mechanisms that type III effectors use to suppress innate immunity are not well understood. Identifying the enzymatic activities of type III effectors and their substrates is essential to identify important components of innate immunity and to improve strategies to control bacterial diseases.

Goal

Objective

Overall hypothesis

Link to preliminary data

Rationale

Our *long-term goal* is to elucidate the molecular basis for suppression of innate immunity by type III effectors. The objective of this application is to identify targets of the *P. syringae* type III effector HopU1, a mono-ADP-ribosyltransferases (ADP-RTs), and to determine its roles in bacterial pathogenesis. *The central hypothesis of the proposed experiments is that the targets of the HopU1 ADP-RT type III effector will be components of innate immunity.* We formulated this hypothesis based on the literature and on our research on other type III effectors as well as our preliminary data showing that HopU1 suppresses outputs of innate immunity. Recently, we have shown that HopU1 can use several *Arabidopsis* RNA-binding proteins as high affinity substrates in *in vitro* ADP-RT assays. Based on our preliminary data, one of these proteins, *At*GRP7, plays a role in innate immunity. A major goal of this application is to elucidate the function of this protein as it relates to innate immunity. We are prepared to undertake the proposed research because we have extensive experience in manipulating type III systems, and we were among the first to report that certain type III effectors suppress innate immunity. In addition, our preliminary identification of HopU1's substrates has positioned us well to perform the experiments described in this application. Our research team includes experts in the following areas: type III secretion systems, proteomics and mass spectrometry, Affymetrix microarrays, plant glycine-rich RNA-binding proteins, and animal pathogen ADP-RTs. This qualified group of investigators will insure that our discoveries are linked to basic concepts of pathogenesis and immunity in both plants and animals.

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Introduction

Mark S. Smeltzer, University of Arkansas

Big picture/ Rele-vance

Staphylococcus aureus is a well-armed opportunistic pathogen that produces a diverse array of virulence factors and causes a correspondingly diverse array of infections. The pathogenesis of S. aureus infections depends on the coordinately-regulated expression of two groups of virulence factors, one of which (surface proteins) allows the bacterium to evade phagocytes and colonize host tissues while the other (extracellular toxins and enzymes) promotes survival and multiplication at a localized site of infection. Our long term goal is to elucidate the regulatory mechanisms controlling expression of these virulence factors as a prerequisite to the development of therapeutic protocols that can be used to attenuate the disease process.

Goal

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Overall hypothesis

Sub-hypotheses and rationale

Short-term goal

Lead-in to Specific Aims

The specific hypothesis behind the proposed research is that the staphylococcal accessory regulator *(sar) is a major regulatory* switch controlling expression of *S. aureus virulence factors. That* hypothesis is based on the following observations. **First, *sar encodes a*** DNA-binding protein (SarA) required for expression of the *agr-encoded* RNAIII regulatory molecule (27). The SarA-dependency of RNAIII expression is important because RNAIII modulates expression of many *S. aureus virulence factors (29).* ***Second, phenotypic comparison of sar*** and *agr mutants indicates that sar also regulates expression of certain S. aureus genes in an agr-independent manner (11, 21). An example of* particular relevance to this proposal is the *S. aureus collagen adhesin* gene (*cna).* ***Third, mutation of sar results in reduced virulence in animal*** models of staphylococcal disease (8, 10, 28). Moreover, as anticipated based on the preceding discussion, *sar/agr double mutants have* reduced virulence even by comparison to *agr mutants (8, 24).* ***Based on* these observations, the experimental focus of this proposal is on the *sar regulatory locus.*** ***The specific aims are designed to provide a*** comprehensive assessment of the *agr-independent regulatory functions* of *sar:*

Specific Aims

Specific Aim 1. We will test the prediction that X…..

1A. We will manipulate X and measure Y …

Our hypothesis predicts …

1B. We will do X assay to determine ….

We expect to find …

Specific Aim 2. To characterize the mechanism of …

2A. We will test whether X happens by Y….

We predict that …

2B. We will test the hypothesis that X happens by carrying out Y methods …

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Specific Aims

Paul Hagerman, University of California, Davis, 5UL1DE019583-04

Aim 1: To develop oligonucleotide-based approaches to reduce FMR1 RNA in vivo.

Experiments will test predictions of our hypothesis that reductions in FMR1 RNA levels in neurons and/or astrocytes will ameliorate the neuropathology of FXTAS. This aim also tests the location (nuclear vs. cytoplasmic) of the cellular pathology caused by expression of the exCGG.

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Specific Aims

Aim 2: To define the timing and reversibility of pathogenic responses to expression exCGG RNA.

Tet-inducible neural cell models will be used to elucidate the time course of pathogenic responses to expression of the exCGG RNA and the extent to which they are reversible, thereby guiding Projects 2 and 3 in the development of new therapeutic interventions.

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Specific Aims

Aim 3: To evaluate the roles of astrocyte and neuronal dysfunction in the pathogenesis of FXTAS.

Biochemical and pharmacological experiments will test our hypothesis, which predicts that loss of normal astrocyte function, possibly impairing glutamate uptake and/or alterations in neuronal glutamate signaling pathways, contribute subtle to severe changes in neuronal morphology and cell loss. These studies will provide mechanistic understanding of clinical interventions with memantine and lithium (Project 3).

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Specific Aims

Boris Striepen, University of Georgia
"Biology of the apicomplexan plastid"

Specific Aim 1: Dissect the mechanism of apicoplast protein import. The bulk of the ~500 apicoplast proteins is nuclear encoded and post-translationally imported across four membranes. We (and others) have described three mechanistically distinct candidate protein translocons that reside in the three inner membranes of complex plastids. In the current funding period we will focus on a newly discovered mechanism that was derived from the ER-associated degradation system (ERAD) of the algal endosymbiont. We will use conditional gene disruptions and complementation assays to establish the importance of individual components and to define the energy source of the translocation process.

Specific Aims

Boris Striepen, University of Georgia
"Biology of the apicomplexan plastid"

Specific Aim 2: Understand the function of the apicoplast ubiquitination pathway. The ER-localized ERAD pathway goes hand in hand with the ubiquitination and subsequent proteasomal degradation of translocated proteins. Our preliminary data indicates that aspects of this protein modification pathway are still present in the apicoplast. What is the enzymatic machinery involved in this process? What are its substrates? And most importantly, what is the molecular function of apicoplast ubiquitination? A combination of genetic and biochemical approaches will be used to answer these important questions.

Specific Aims

Boris Striepen, University of Georgia
"Biology of the apicomplexan plastid"

Specific Aim 3: Discover a comprehensive set of apicoplast proteins and characterize their function. Mining comparative and functional genomic information we have assembled an extensive list of proteins for which we hypothesize a role in apicoplast biology. We will establish the localization of their protein products for a comprehensive set of these candidate genes by epitope tagging. In the previous funding period we have found conditional null mutants to be highly informative to study apicoplast protein function and we have developed phenotypic assays to detect defects in apicoplast protein import, apicoplast division, and apicoplast metabolism. We will apply this genetic approach to a prioritized list of validated candidates. To increase the throughput of our analyses we will develop and test a new mutagenesis approach based on promoter replacement.

Specific Aims: Adding Sub-Aims

1. ***Correlate*** ***the production of each sar transcript with the production of functional SarA.*** The only recognized protein product of the sar locus is the SarA DNA-binding protein. However, Northern blot analysis reveals three sar transcripts (sarA, sarB and sarC), all of which include the entire *sarA* gene. Expression of each transcript is growth-phase dependent. The functional significance of this differential regulation will be assessed by correlating the production of each transcript with the production and activity of SarA.

A. The temporal production of SarA will be assessed by Western blot of *S. aureus* whole cell extracts with an affinity-purified anti-SarA antibody.

B. The DNA-binding activity of SarA will be assessed by electrophoretic mobility shift assays (EMSA) using whole cell extracts and DNA fragments known to include SarA-binding sites (e.g. *cis* elements upstream of the *agr* P2 and P3 promoters).

C. The function of SarA as a transcriptional activator will be assessed *in vivo* using transcriptional fusions between each of the *agr* promoters and a promoter-less *xylE* reporter gene.

Specific Aims: Adding Sub-Aims

Specific Aim 1A. Does the fMRI response at the trailing edge of a moving object reveal a deblurring mechanism? Based on pilot results, we hypothesize that parametrically correlating psychophysical data to fMRI responses will reveal the neural mechanism of deblurring in V1 and/or MT+ (Experiments D. 1B-D.1E, & D.1G). TMS will be used to stringently test whether the psychophysically measured modulations in perceived position are causally linked to the neural responses revealed in the fMRI studies (Experiment D.1H).

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Specific Aims: Adding Sub-Aims

Specific Aim 1B. The precision of position coding in MT+: Some models of localization suggest that position and motion information are integrated in MT+. On the other hand, MT+ is thought to be only coarsely retinotopic. To reconcile these differing views, we will (1) test whether MT+ plays a key role in coding object position (not just motion) and (2) establish the spatial resolution at which MT+ is able to code position (Experiment D.1C).

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Specific Aims – what is wrong?

Aim X. To use targeted gene replacement to create a BRCA1$^{-/-}$ knockout mouse.

Aim X. To analyze gene expression profiles using microarray analysis in normal vs. cancerous prostate cells.

Aim X. To overexpress several components of the telomere enzyme in *S. cerevisiae* and measure DNA repair efficiency at telomere ends.

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Specific Aims – what is wrong?

Aim 1. Show that p53 is upregulated in a chronic inflammation mouse model.

Aim 2. Demonstrate that downregulation of p53
using siRNA decreases inflammation.

Aim 3. Characterize additional p53 binding targets due to upregulation in response to chronic inflammation.

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Diagram Specific Aims

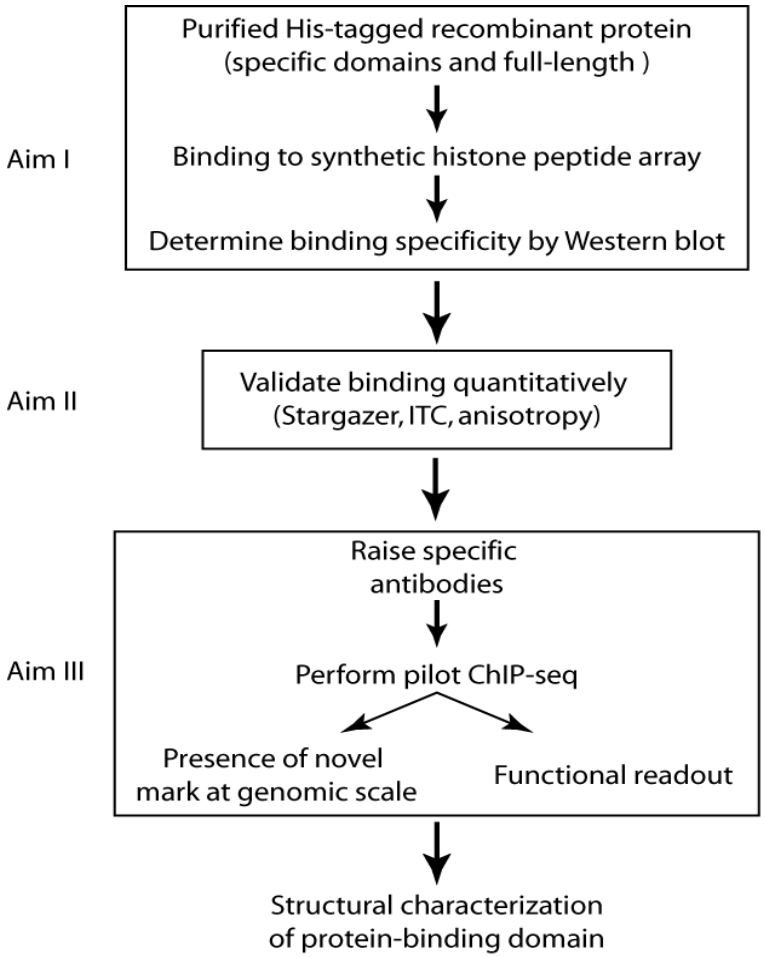

Fig. 1. Schematic flowchart for the current proposal. The main steps along each specific aim are indicated. See text for details.

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Diagram Specific Aims

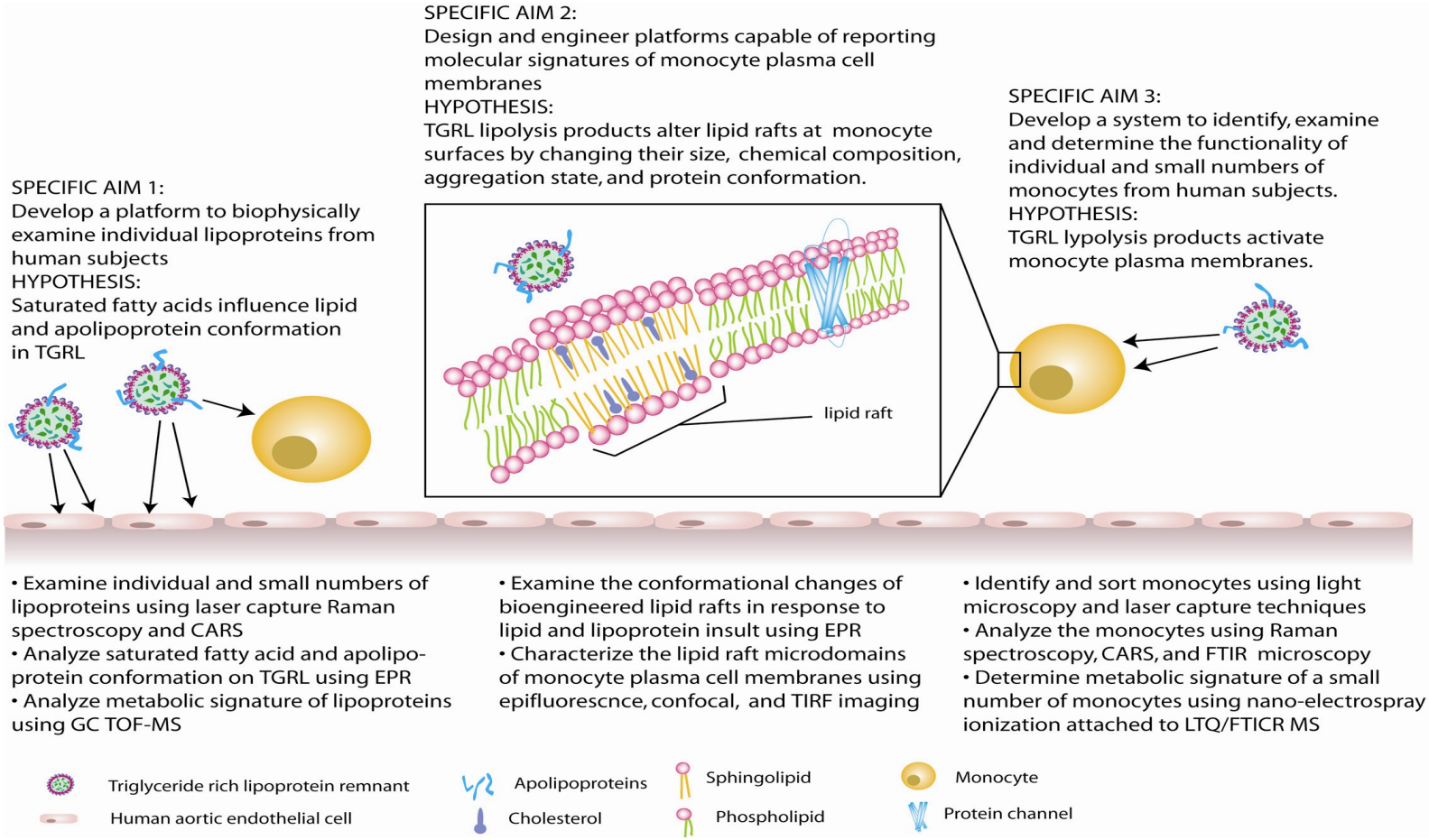

Figure 1. Research overview for the Bioengineering Research Partnership on lipid-membrane interactions.

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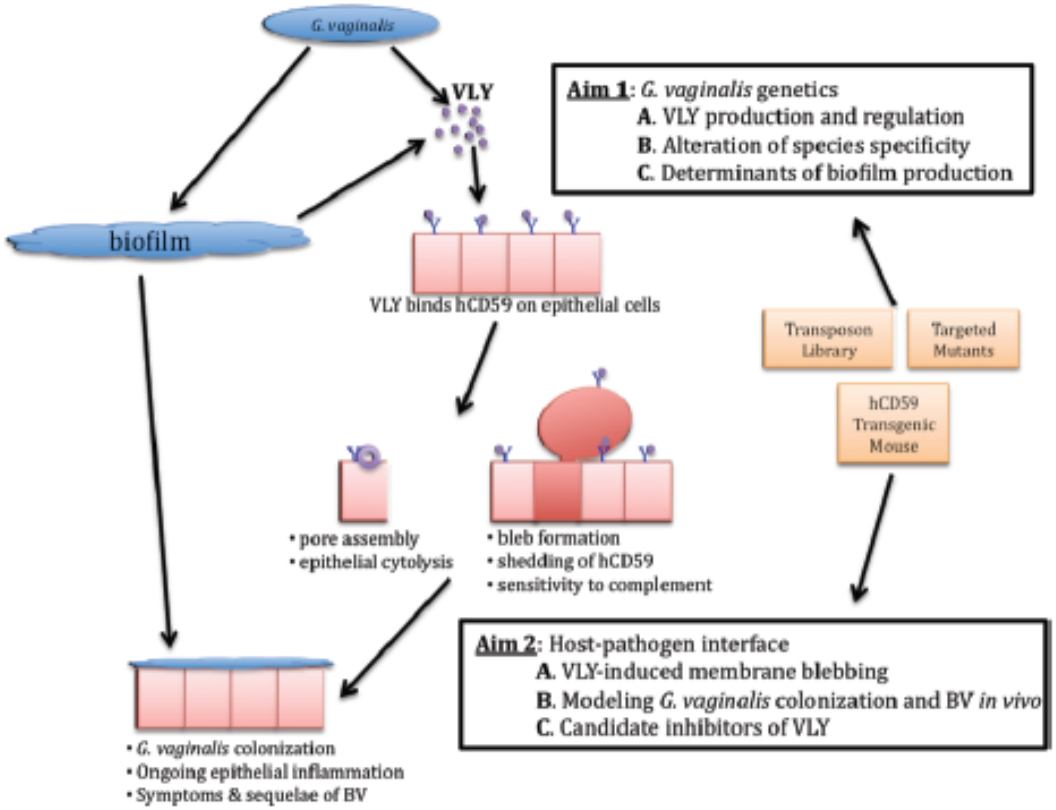
Diagram Specific Aims



Ratner, AJ. R01 HD061371-01, *"Gardnerella vagina/is:* toxin production and pathogenesis"

Look at Funded Specific Aims

PI and Grantee Institution	Application and Summary Statement
Dr. James R. Alfano University of Nebraska at Lincoln	Resubmission of an unsolicited application: Application 1-R01-AI069146-01A2: Suppression of innate immunity by an ADP-ribosyltransferase type III effector
Dr. George Louis Drusano Ordway Research Institute, Inc.	Application responding to a request for applications: Application 1-R01-AI079729-01: Resistance Suppression for Influenza Virus With Combination Chemotherapy
Dr. Christopher D. Huston (new investigator) University of Vermont	Resubmission of an unsolicited application: Application 1-R01-AI072021-01A2: Molecular Mechanism of *Entamoeba histolytica* Phagocytosis
Dr. Michael G. Rossmann Purdue University at West Lafayette	Resubmission of an unsolicited application: Application 1-R01-AI076331-01A1: Structure and Function of Flaviviruses

Look at http://funding.niaid.nih.gov/researchfunding/grant/pages/appsamples.aspx

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How to develop and write an NIH grant

Rita Balice-Gordon, Ph.D.
Professor
Dept. of Neuroscience

How to become funded

- Idea
- Commitment
- Grant writing skills

Idea: how to develop one

- Be knowledgable
  - Extensively read existing literature
  - Where is the current cutting edge of knowledge?
- Be thoughtful
  - Devote time to "just" thinking
  - Think in question format: formally write out every question you'd like to ask that's even remotely related to your project
  - Think in experiment format: formally write out every possible experiment you should do or you dream about doing – with no consideration of money, expertise or equpiment
  - Think in hypothesis format: formally write out all of the hypotheses related to your project
- Be creative
  - Borrow tools and approaches from other fields
  - Combine these in new and compelling ways
- Be open to feedback and criticism
  - Share your ideas with colleagues before you start writing
  - Learn to accept criticism – it's not personal

Commitment

- Passion
- Attitude
  - I can't → - I can and I will
  - I don't have time → - I will reorder my priorities
  - There's too much competition → - I welcome the chance to compete
  - It's good as it is now → - It can always be better
  - I'll submit now and "get in line" → - I won't submit until it's the very best grant I can write
- Time
  - Lead time – *how much time do you think it takes to prepare a 25 page grant submission?*
  - Quality time

Grant writing skills

- There's one and only one key point
  - You have to sell your ideas to reviewers
  - You have to make the Reviewer your advocate in the Study Section (more on that later)
- How?

How to sell your ideas to the Reviewer

A successful salesperson

- Has something special to offer (significance and importance of work to the field)
- Makes a good first impression (Specific Aims Page)
- Is well prepared and knowledgable (B+S section)
- Has appropriate credentials (BioSketch)
- Provides supporting documentation (Preliminary data; published papers)
- Delivers a clear message than can be understood by a knowledgable person without specialized background (Research plan)
- Has appropriate endorsements (Letters of Collaboration from colleagues)
- Is persistent!!!

Abstract

- A concise summary of the question/hypothesis, aims and their SIGNIFICANCE

- Text boxes taken from Dr. Erfei Bi, Associate Professor, Dept. of Cell Biology and Development, Univ. Penn SOM

Signaling mechanisms in cell polarity in yeast

My long-term objective is to use the genetically tractable eukaryote Saccharomyces cerevisiae to determine how Cdc42p, an evolutionarily conserved GTPase, controls the organization of the actin cytoskeleton and of the septins. Recent work suggests that Cdc42p controls the actin organization by two parallel pathways: one involving Msb3p, Msb4p, and Bni1p; the other involving Gic1p, Gic2p, and Bem4p. In the present studies, these pathways will be explored further, focusing initially on the roles of Msb3p and Msb4p.

Msb3p and Msb4p, a pair of highly homologous proteins in yeast, play a redundant role in linking Cdc42p to the actin cytoskeleton. They belong to a family of proteins that includes the Drosophila cell adhesion molecule, Pollux, and the human oncoprotein, Tre17. Thus, studying the function of Msb3p and Msb4p will shed significant light on the function of this family of proteins. Specifically, research in this proposal will address the following questions: What proteins link Msb3p and Msb4p to the upstream Cdc42p, and to the downstream actin cytoskeleton? How are the proteins in the Msb-mediated pathways organized at the molecular level? What are the functions of the putative domains in Msb3p and Msb4p (the highly conserved PTM domain, the membrane-spanning domains, and a lipid modification site)? How are the localization of Msb3p and Msb4p regulated in the cell cycle? These questions will be answered with a combination of genetic, cytological, and biochemical approaches. To expand our hypothesis, additional genes that are involved in regulating Cdc42p activity or a specific effector pathway will be identified by genetic screens, and affinity chromatography coupled with tandem mass spectrometry.

Homologs of Cdc42p are involved in diverse cellular processes, such as cell polarity, cell migration, and cell growth control. In addition, deregulation of Cdc42p activity in mammals is associated with serious diseases, such as cancer. Thus, studying the signaling mechanisms of Cdc42p in yeast represents an important bridge between basic and clinical sciences.

Specific Aims section

- The single most important section in the grant
  - It's the master plan for the rest of the proposal
  - You engage or lose the Reviewer on this page
- It's the most difficult section to write
  - The logic of each aim must be compelling
  - The answers must be important to the field
- Write Aims that you are excited about!

Specific Aims section

- Whenever possible – test a hypothesis in the specific aim title
  - You want the Reviewer to know that your work is hypothesis driven
  - Don't make the Reviewer work to figure out what the hypothesis is
- The goal of the aim should be to understand mechanism – even if the experiments are largely descriptive
- 3 – 4 Specific Aims for a 4 to 5 year grant – each aim is a paper, or is a significant part of a paper
- The Specific Aims should be detailed but far reaching – the Aims should not be a list of experiments

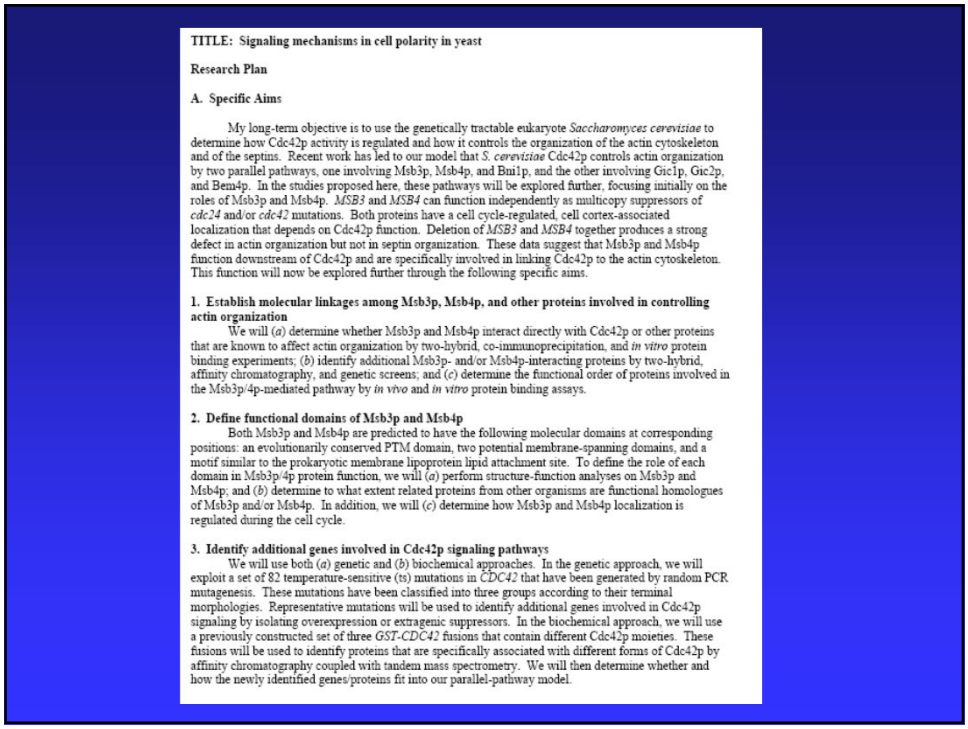
Specific Aims - Examples

Okay:

Specific Aim 1: To test the hypothesis that neurons in the GluR1 knockout mouse will have delayed dendritic maturation.

Better:

Specific Aim 1: To test the hypothesis that GluR1 signaling is necessary for dendritic maturation. (or is sufficient).



TITLE: Signaling mechanisms in cell polarity in yeast

Research Plan

A. Specific Aims

My long-term objective is to use the genetically tractable eukaryote *Saccharomyces cerevisiae* to determine how Cdc42p activity is regulated and how it controls the organization of the actin cytoskeleton and of the septins. Recent work has led to our model that *S. cerevisiae* Cdc42p controls actin organization by two parallel pathways, one involving Msb3p, Msb4p, and Bni1p, and the other involving Gic1p, Gic2p, and Bem4p. In the studies proposed here, these pathways will be explored further, focusing initially on the roles of Msb3p and Msb4p. *MSB3* and *MSB4* can function independently as multicopy suppressors of *cdc24* and/or *cdc42* mutations. Both proteins have a cell cycle-regulated, cell cortex-associated localization that depends on Cdc42p function. Deletion of *MSB3* and *MSB4* together produces a strong defect in actin organization but not in septin organization. These data suggest that Msb3p and Msb4p function downstream of Cdc42p and are specifically involved in linking Cdc42p to the actin cytoskeleton. This function will now be explored further through the following specific aims.

1. Establish molecular linkages among Msb3p, Msb4p, and other proteins involved in controlling actin organization

We will (*a*) determine whether Msb3p and Msb4p interact directly with Cdc42p or other proteins that are known to affect actin organization by two-hybrid, co-immunoprecipitation, and *in vitro* protein binding experiments; (*b*) identify additional Msb3p- and/or Msb4p-interacting proteins by two-hybrid, affinity chromatography, and genetic screens; and (*c*) determine the functional order of proteins involved in the Msb3p/4p-mediated pathway by *in vivo* and *in vitro* protein binding assays.

2. Define functional domains of Msb3p and Msb4p

Both Msb3p and Msb4p are predicted to have the following molecular domains at corresponding positions: an evolutionarily conserved PTM domain, two potential membrane-spanning domains, and a motif similar to the prokaryotic membrane lipoprotein lipid attachment site. To define the role of each domain in Msb3p/4p protein function, we will (*a*) perform structure-function analyses on Msb3p and Msb4p; and (*b*) determine to what extent related proteins from other organisms are functional homologues of Msb3p and/or Msb4p. In addition, we will (*c*) determine how Msb3p and Msb4p localization is regulated during the cell cycle.

3. Identify additional genes involved in Cdc42p signaling pathways

We will use both (*a*) genetic and (*b*) biochemical approaches. In the genetic approach, we will exploit a set of 82 temperature-sensitive (ts) mutations in *CDC42* that have been generated by random PCR mutagenesis. These mutations have been classified into three groups according to their terminal morphologies. Representative mutations will be used to identify additional genes involved in Cdc42p signaling by isolating overexpression or extragenic suppressors. In the biochemical approach, we will use a previously constructed set of three *GST-CDC42* fusions that contain different Cdc42p moieties. These fusions will be used to identify proteins that are specifically associated with different forms of Cdc42p by affinity chromatography coupled with tandem mass spectrometry. We will then determine whether and how the newly identified genes/proteins fit into our parallel-pathway model.

Specific Aims: Dos

- Write your Aims early – some may fall apart as you design a plan to test them or discuss them with colleagues
- Try to limit this section to one page – it's a roadmap to the rest of the proposal and it must include the logic behind your aims.
- Don't assume your Reviewer is an expert in your particular area – so write Aims for a non-expert compared to the rest of the proposal

Specific Aims: Don'ts

- Don't state a hypothesis that you cannot actually test with the experiments you are proposing
- Avoid using phrases like: To correlate… To describe… To develop; these help get your grant pegged as "too descriptive"
- Avoid wishy-washy, passive tense, or flowery language – instead write your aims in active form with strong meaningful verbs
- Don't write aims that can be viewed as "a fishing expedition" – microarray experiments, expression cloning, etc.

Background and Significance

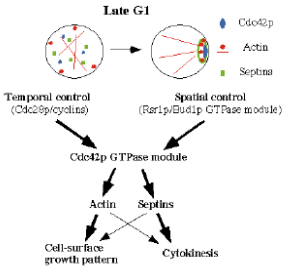
- Background
  - Should lead the reader to each question or hypothesis that you're testing in each aim
- Significance
  - State this explicitly
  - This section must explain why the Study Section should fund your proposal rather than the next one
  - What is the "value added" to your field if you're able to do the work?

B. Background and Significance

Cell polarity can be simply defined as the asymmetric organization of cellular components, including plasma membrane proteins, organelles, and cytoskeletal elements. The generation of asymmetry or functional domains within the cell plays a crucial role in many biological processes and is essential for development and differentiation (20, 30). However, the molecular mechanisms for establishing the axis of cell polarity are not well understood in any system. We are using the budding yeast *Saccharomyces cerevisiae* to study how the actin cytoskeleton is polarized at the molecular level because of the many experimental advantages of this organism, including its genetic tractability. In addition, the behavior of the actin cytoskeleton during the *S. cerevisiae* cell cycle has been well characterized and can easily be monitored (2, 44, 50), thus providing a convenient assay for examining the effects of mutations on actin organization.

Parallel studies in many systems have firmly established that Cdc42p, an evolutionarily conserved GTPase, plays a central role in the polarized organization of the actin cytoskeleton in diverse organisms ranging from yeast to humans. During the last few years, accumulating evidence has suggested that Cdc42p also plays a role in other cellular processes. In this section, I will briefly review what we know and what we do not know about the function of Cdc42p in yeast and other systems; then, I will discuss the significance of my proposed studies.

The diagram below summarizes our current understanding of the morphogenetic pathway in *S. cerevisiae* (Fig. 1).



Cell polarity in yeast

In budding yeast, newly divided cells grow isotropically in G1 until the cell reaches a critical cell size, then the master cell cycle control system, comprising Cdc28p kinase and the G1 cyclins, is activated

Preliminary Studies

- In order of Specific Aims
- You don't have to know the outcome of each experiment before the grant is submitted
- You DO have to:
  - Show that you can perform all of the necessary techniques and methods (Letters of Collaboration)
  - You are committed to this area of research and are off and running
  - New techniques are feasible, reliable and yield interpretable data

C. Preliminary Studies

Introduction. Most of the studies described below were performed by me, with some help from two undergraduate students, while I was a postdoctoral fellow in John Pringle's laboratory at the University of North Carolina at Chapel Hill. The initial identification and characterization of *MSB3* and *MSB4* are described in the accompanying paper (9). Since January, 1998, I have established my own laboratory at the University of Pennsylvania School of Medicine. I am continuing to explore the molecular role of Msb3p and Msb4p in linking Cdc42p to the actin cytoskeleton. In addition, we are undertaking genetic and biochemical approaches to identify additional genes involved in Cdc42p signaling. This section contains three sets of results that form the basis for the three Specific Aims.

1. Msb3p and Msb4p are specifically involved in linking Cdc42p to the actin cytoskeleton

Genetic interaction between *MSB3* or *MSB4* and *CDC42*. *MSB3* was isolated as a gene that can serve as a multicopy suppressor of a particular *cdc24* allele (*cdc24-11*) in conjunction with multicopy *CDC42*. In addition, multicopy *MSB3* by itself suppresses a *cdc42*^{ts} mutation, suggesting that Msb3p positively regulates Cdc42p function. A close homolog, *MSB4*, was identified by database searching. Msb3p and Msb4p are 50% identical in amino acid sequence over the entire proteins. Like *MSB3*, multicopy *MSB4* and *CDC42* together suppressed *cdc24-11*, suggesting that Msb3p and Msb4p are functional homologs.

Msb3p and Msb4p are functionally redundant and are specifically involved in actin organization. Deletion of *MSB3* or *MSB4* alone did not produce any obvious phenotype. However, deletion of both genes together resulted in slow cell growth, particularly at lower temperatures. In addition, a large proportion of the double mutant cells became larger and rounder than normal (Fig. 3), with F-actin randomly distributed in the cell cortex; this resembles the phenotype of a *cdc42*^{ts} mutant at the nonpermissive temperature. These data suggest that Msb3p and Msb4p play a redundant role in actin organization. In addition, they support the genetic conclusion that Msb3p and Msb4p positively regulate Cdc42p function.

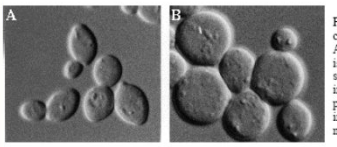


Fig. 3. Morphological defects caused by *msb3* and *msb4* deletions. A wild-type strain (A) and an isogenic *msb3 msb4* double mutant strain (B) were grown exponentially in rich medium at 23°C, then processed for Differential-interference-contrast (DIC) microscopy.

To ask whether Msb3p and Msb4p function as general regulators of Cdc42p (regulate the ratio of GTP/GDP-bound Cdc42p) or as specific effectors involved in actin organization, the following logic was applied: Cdc42p function is required for the polarized organization both of the actin cytoskeleton and of the septins at the beginning of the cell cycle. However, the organization of these two cytoskeletal elements is independent of each other (3, 35). Thus, if Msb3p and Msb4p are general regulators of Cdc42p, deletion of these genes should affect the organization of both cytoskeletal elements; if Msb3p and Msb4p function as a part of the effector pathway that links Cdc42p to the actin cytoskeleton, deletion of both genes should not affect septin organization. The latter result was obtained: the septins were able to organize normally in the fraction of *msb3 msb4* double mutant cells that had lost actin organization (a representative cell is shown in Fig. 4), suggesting that Msb3p and Msb4p function downstream of Cdc42p and are specifically involved in linking Cdc42p to the actin cytoskeleton.

Experimental Plan

- Specific Aims are fleshed out with the actual experimental approach
  - Rationale (1 paragraph) -- logic
  - Experiments – how
    - CONTROLS (positive and negative)
  - Analysis and Interpretation – what will results mean?
  - Pitfalls and Alternative Approaches
  - Detailed Methods

D. Research Design and Methods

Training of the applicant. I have extensive training in genetics, cell biology, and molecular biology from my prior work in Joe Lutkenhaus's laboratory at the University of Kansas Medical Center and in John Pringle's laboratory at the University of North Carolina. When I was a graduate student, I used a combination of approaches to study bacterial cell division, including co-immunoprecipitation, cross-linking, and immunoelectronmicroscopy. During my postdoctoral training, I have been highly experienced in yeast genetics and cell biology. With specific questions in mind, I have performed several successful genetic screens. In addition, I have used a variety of microscopy techniques including video microscopy, and I have also done affinity purification and protein extraction experiments. Thus, I believe that the experiments proposed here can be efficiently carried out in my laboratory, which is already well equipped by virtue of my start-up package from the University of Pennsylvania.

Aim 1. Establish molecular linkages among Msb3p, Msb4p, and other proteins involved in controlling actin organization

One of the central issues in polarity establishment is how positional information is communicated from Cdc42p in the cell cortex to the actin cytoskeleton. As discussed above, genetic and cytological evidence suggests that *S. cerevisiae* Cdc42p controls actin organization through two parallel pathways, one involving Msb3p, Msb4p, and Bni1p, and the other involving Gic1p, Gic2p, and Bem4p. However, many important questions remain unanswered: How do Msb3p and Msb4p interact with other components in the same pathway biochemically? Are there additional factors that function upstream or downstream of Msb3p and Msb4p to link Cdc42p to the actin cytoskeleton? What is the functional order among the components in each pathway? How are the parallel pathways organized at the molecular level? Given the power of yeast genetics and the well characterized actin behavior during the yeast cell cycle, the functional output of each physical interaction can easily be assessed by monitoring the effects of alterations in that particular interaction on actin organization *in vivo*. Answers to all the questions above are essential for elucidation of the molecular mechanisms by which Cdc42p controls actin organization.

Question #1: Do Msb3p and Msb4p interact directly with Cdc42p and/or with other proteins that are known to affect actin organization? Based on the parallel-pathway model, the simplest prediction is that Msb3p and Msb4p interact directly with the GTP-bound form of Cdc42p, and perhaps with Bni1p, but do not interact with Gic1p, Gic2p, or Bem4p. It is also possible that Msb3p and Msb4p are linked to Cdc42p through other proteins that are known to affect actin organization, such as Bem1p, Bem2p, Boi1p, Boi2p, Rho3p, or Rho4p.

Methods: (i) Two-hybrid: We have cloned full-length Msb3p and Msb4p into the bait plasmid pEG202 (87), as well as into a prey plasmid, a modified version of pJG4-5 (29, 36). These constructs will be assayed for their interactions with various forms of Cdc42p (the wild-type, constitutively active, and constitutively inactive forms), Bni1p, Gic1p, Gic2p, Bem4p, Bem1p, Bem2p, Boi1p, Boi2p, Rho3p, and Rho4p. Most of the relevant constructs are already available, and preliminary assays have shown that full-length Msb3p and Msb4p have a weak interaction with the constitutively activated forms of Cdc42p [$Cdc42p^{G12V,C188S}$ and $Cdc42p^{Q61L,C188S}$ (the purpose of the C188S mutation in the CAAX box is to prevent the membrane localization of Cdc42p so that a two-hybrid interaction in the nucleus is possible)]. These weak interactions were observed only when *MSB3* or *MSB4* was under inducible promoter control in the prey plasmid. No interaction was observed between full-length Msb3p or Msb4p and full-length Bem1p. The other genes listed above have not yet been tested. The full matrix of potential two-hybrid interactions among these genes will be thoroughly examined and the results will be integrated with those from other approaches in an attempt to define the proteins with which Msb3p and Msb4p directly interact.

Anticipated Results/Pitfalls: Both Msb3p and Msb4p are predicted to contain at least one membrane-spanning domain, which may explain why a weak interaction between the full-length Msb3p or Msb4p and the constitutively active form of Cdc42p was observed only when the expression of *MSB3* or *MSB4* was induced to a high level in the prey plasmid (perhaps, a small amount of the full-length protein can get into the nucleus under such conditions). Even if Msb3p and Msb4p are indeed membrane proteins (to be investigated under Specific Aim 2), the two-hybrid approach can still be applied by using fragments of Msb3p or Msb4p that do not contain the possible membrane-spanning domain. Even for nonmembrane proteins, it has been well documented that in some cases, a fragment of the protein shows stronger interaction with the partner than does the full-length protein. The advantage of the two-hybrid approach is that many potential interactions can be assayed easily in a short time. However, at best, the results from two-hybrid assays can only indicate that two proteins are capable of interacting with each other (directly or through a bridging protein) in the nucleus when overexpressed.

Other grant parts

E. Human Subjects

F. Vertebrate Animals

G. Literature Cited

H. Consortium/Contractual Arrangements

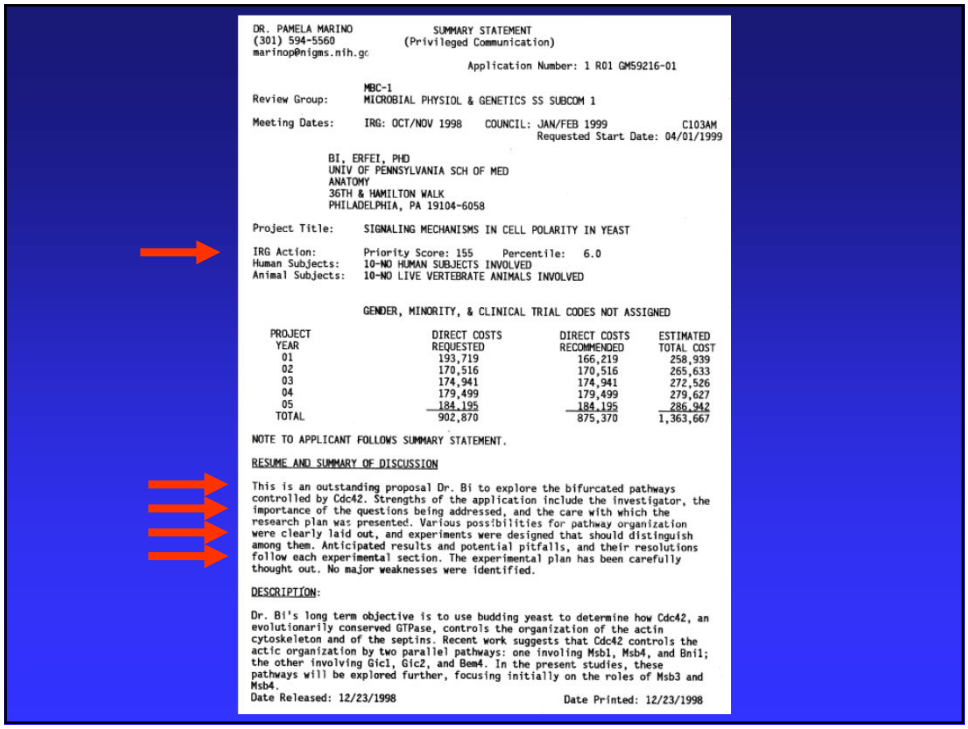
I. Consultants

You have a draft…now what?

- Rewrite.
  - Read each sentence ALOUD. Can it be made simpler? Less wordy? More compelling?
  - The only good writing is REWRITING.
- Get feedback from other scientists – in and somewhat tangential to your field
  - Timing
  - Accepting criticism
  - "Pay it forward" principle
- Repeat above.

Other Important Issues

- Page requirements
- Font size and line spacing
- SPACING OF TEXT SECTIONS
- Embed figures into the text. Include a brief, clear legend.
- Figure must be absolutely clear/visible to the Reviewer – include color pages and mark these copies as "Color Figures for Reviewer."
- Learn how to use MS Word
- Spelling and grammar – ZERO TOLERANCE for sloppy mistakes.



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SUMMARY STATEMENT
(Privileged Communication)

Application Number: 1 R01 GM59216-01

MBC-1
Review Group: MICROBIAL PHYSIOL & GENETICS SS SUBCOM 1

Meeting Dates: IRG: OCT/NOV 1998 COUNCIL: JAN/FEB 1999 C103AM
Requested Start Date: 04/01/1999

BI, ERFEI, PHD
UNIV OF PENNSYLVANIA SCH OF MED
ANATOMY
36TH & HAMILTON WALK
PHILADELPHIA, PA 19104-6058

Project Title: SIGNALING MECHANISMS IN CELL POLARITY IN YEAST

IRG Action: Priority Score: 155 Percentile: 6.0
Human Subjects: 10-NO HUMAN SUBJECTS INVOLVED
Animal Subjects: 10-NO LIVE VERTEBRATE ANIMALS INVOLVED

GENDER, MINORITY, & CLINICAL TRIAL CODES NOT ASSIGNED

PROJECT YEAR	DIRECT COSTS REQUESTED	DIRECT COSTS RECOMMENDED	ESTIMATED TOTAL COST
01	193,719	166,219	258,939
02	170,516	170,516	265,633
03	174,941	174,941	272,526
04	179,499	179,499	279,627
05	184,195	184,195	286,942
TOTAL	902,870	875,370	1,363,667

NOTE TO APPLICANT FOLLOWS SUMMARY STATEMENT.

RESUME AND SUMMARY OF DISCUSSION

This is an outstanding proposal Dr. Bi to explore the bifurcated pathways controlled by Cdc42. Strengths of the application include the investigator, the importance of the questions being addressed, and the care with which the research plan was presented. Various possibilities for pathway organization were clearly laid out, and experiments were designed that should distinguish among them. Anticipated results and potential pitfalls, and their resolutions follow each experimental section. The experimental plan has been carefully thought out. No major weaknesses were identified.

DESCRIPTION:

Dr. Bi's long term objective is to use budding yeast to determine how Cdc42, an evolutionarily conserved GTPase, controls the organization of the actin cytoskeleton and of the septins. Recent work suggests that Cdc42 controls the actic organization by two parallel pathways: one involing Msb1, Msb4, and Bni1; the other involving Gic1, Gic2, and Bem4. In the present studies, these pathways will be explored further, focusing initially on the roles of Msb3 and Msb4.

Date Released: 12/23/1998 Date Printed: 12/23/1998

Responding to the Reviews

- Read the reviews.
- Get over your disappointment and anger.
- Don't take it personally.
- Respect the Reviewers, their Reviews, and the process.
- Take them seriously – often they are right.
- Make a list of the major and minor issues and respond first to the major ones.
- Directly respond to the criticisms with positive responses. If the Reviewer misunderstood and is thus wrong -- it's your fault, not theirs!
- So, show them why, using facts, logic, additional explanation, references, etc.
- Do not NOT address one of the issues, even the most minor one, that is raised by a Reviewer – especially if more than one Reviewer mentions it.
- Don't send the same grant back. You must show progress, evolution of your thinking, etc.
- Don't include anything so far out that can raise new questions if your score is close.

NIH's new electronic grant application process

Date: Tue, 13 Dec 2005 17:00:00 -0500
Reply-To: PennERA@POBOX.UPENN.EDU
Sender: PennERA Proposal Tracking Investigators <ERA_PT_INVESTIGATORS@LISTS.UPENN.EDU>
From: PennERA@POBOX.UPENN.EDU
Subject: NIH/Grants.gov Webcast Update
To: ERA_PT_INVESTIGATORS@LISTS.UPENN.EDU
X-Spam-Checker-Version: SpamAssassin 3.0.0 (2004-09-13) on pobox.upenn.edu
X-Spam-Status: No, score=-10.6 required=5.0 tests=ALL_TRUSTED,HTML_30_40, HTML_MESSAGE,MIME_HTML_ONLY,NO_REAL_NAME autolearn=disabled version=3.0.0
X-Spam-Level:

TITLE: NIH's New Electronic Grant Application Process and the SF424 (R&R)

PURPOSE: By May 2007 all research grant applications for NIH will have to be submitted electronically through Grants.gov using the SF424 Research & Related (R&R) form set. This training session, geared toward the applicant community, will provide an overview of NIH's transition plans, the submission process and the new form set. A question and answer session will follow the formal presentations.

WHEN & WHERE: 2 Sessions Available DUNLOP AUDITORIUM, ground floor, Stemmler Hall
Wednesday, January 11, 2006, 8:30 AM to 12:00 PM EST
Wednesday, January 11, 2006, 12:30 PM to 4:00 PM EST

Both the morning and afternoon sessions will also be available for remote viewing via VideoCast, NIH's streaming video service. For more information OR to register to view this program on your desktop:

http://era.nih.gov/training/ElectronicSubmission/

IF YOU ARE PLANNING TO VIEW THE WEBCAST IN DUNLOP AUDITORIUM, IT IS NOT NECESSARY TO REGISTER.

On line resources for grant writing

- Visit the *Advance* faculty professional development web site at www.med.upenn.edu/fapd/advance and view the following materials on the research page:
  - All About Grants tutorial on developing R01 grant applications produced by the NIAID at the NIH http://www.niaid.nih.gov/ncn/grants/default.htm
    CHECKLIST – very helpful
  - Common Pitfalls of Grant Preparation
    PowerPoint with synchronized voice by Dr. Ann Kennedy, Professor of Research Oncology at Penn School of Medicine
- Some information taken from "Grantsmanship workshop: how to develop a fundable research proposal," T. Bray, Ph.D., Dean, Oregon State Univ. College of Health and Human Sciences

Uses short sentences, is neat, is clean with no typographical errors. Uses bullets and numbered lists for effective organization. No header or footer, since those are added automatically later. Stays within page limit **(in original version, prior to annotation.)**

SPECIFIC AIMS

Staphylococcus aureus is a well-armed opportunistic pathogen that produces a diverse array of virulence factors and causes a correspondingly diverse array of infections. The pathogenesis of *S. aureus* infections depends on the coordinately-regulated expression of two groups of virulence factors, one of which (surface proteins) allows the bacterium to evade phagocytes and colonize host tissues while the other (extracellular toxins and enzymes) promotes survival and multiplication at a localized site of infection. Our long term goal is to elucidate the regulatory mechanisms controlling expression of these virulence factors as a prerequisite to the development of therapeutic protocols that can be used to attenuate the disease process. **The specific hypothesis behind the proposed research is that the staphylococcal accessory regulator *(sar)* is a major regulatory switch controlling expression of *S. aureus* virulence factors.** That hypothesis is based on the following observations. **First**, *sar* encodes a DNA-binding protein (SarA) required for expression of the *agr*-encoded RNAIII regulatory molecule (27). The SarA-dependency of RNAIII expression is important because RNAIII modulates expression of many *S. aureus* virulence factors (29). **Second**, phenotypic comparison of *sar* and *agr* mutants indicates that *sar* also regulates expression of certain *S. aureus* genes in an *agr*-independent manner (11, 21). An example of particular relevance to this proposal is the *S. aureus* collagen adhesin gene (*cna*). **Third**, mutation of *sar* results in reduced virulence in animal models of staphylococcal disease (8, 10, 28). Moreover, as anticipated based on the preceding discussion, *sar/agr* double mutants have reduced virulence even by comparison to *agr* mutants (8, 24). **Based on these observations, the experimental focus of this proposal is on the *sar* regulatory locus.** The specific aims are designed to provide a comprehensive assessment of the *agr*-independent regulatory functions of *sar*:

Specific aims start with the background for the informed non-expert, writing at about the level of *Scientific American*. Gives summary for non-primary reviewers. Puts less technical information first.

Hypothesis is easy to locate in bold type and includes health importance of the project. Provides three reasons for hypothesis, with references.

1. Correlate the production of each sar transcript with the production of functional SarA. The only recognized protein product of the *sar* locus is the SarA DNA-binding protein. However, Northern blot analysis reveals three *sar* transcripts (sarA, sarB and sarC), all of which include the entire *sarA* gene. Expression of each transcript is growth-phase dependent. The functional significance of this differential regulation will be assessed by correlating the production of each transcript with the production and activity of SarA.

A. The temporal production of SarA will be assessed by Western blot of *S. aureus* whole cell extracts with an affinity-purified anti-SarA antibody.

Scope of research is limited to three specific aims listed in bold and followed by a brief description of how each aim will be accomplished. These aims are the steps designed to prove the hypothesis.

B. The DNA-binding activity of SarA will be assessed by electrophoretic mobility shift assays (EMSA) using whole cell extracts and DNA fragments known to include SarA-binding sites (e.g. *cis* elements upstream of the *agr* P_2 and P_3 promoters).
C. The function of SarA as a transcriptional activator will be assessed *in vivo* using transcriptional fusions between each of the *agr* promoters and a promoter-less *xylE* reporter gene.

Lists give guideposts to reviewers; indents and bold add readability.

2. Characterize the mechanism of sar-mediated regulation of the S. aureus collagen adhesin gene (cna). We have established that *sar* is the primary regulatory element controlling *cna* transcription and that this effect involves a direct interaction between SarA and *cis* elements upstream of *cna*. However, unlike SarA binding to the *agr* promoter region, SarA binding represses *cna* transcription. We will correlate the production of each *sar* transcript with the production of SarA and with the regulation of *cna* transcription. We will also define the *cis* elements upstream of *cna* that constitute the SarA DNA-binding target.

A. Complementation of the *cna* transcriptional defect will be done by introducing plasmids encoding the sarA, sarB or sarC transcripts into a *cna*-positive *sar* mutant. Once the SarA-binding site upstream of *cna* has been defined (see below), the complementation studies will be correlated with SarA binding to *cis* elements upstream of *cna*.
B. The SarA DNA-binding site(s) upstream of *cna* will be localized by EMSA using purified SarA. The specific binding site(s) will be identified by DNA footprinting and characterized by EMSA using *cna* sequence variants and purified SarA.
C. The *in vivo* significance of SarA binding will be assessed using transcriptional fusions between sequence variants of the *cis* elements upstream of *cna* and a promoter-less *xylE* reporter gene.

3. Identifify S. aureus virulence factor genes under the direct control of SarA. The scope of SarA as a regulatory protein is not well-defined because the identification of SarA targets has been restricted by the availability of gene probes and/or appropriate phenotypic assays. Our successful purification of SarA in a form capable of binding appropriate DNA targets (e.g. *cis* elements upstream of *agr* and *cna*) will allow us to define the DNA determinants required for SarA binding using a functional selection. We will then identify SarA binding sites within the *S. aureus* genome and evaluate SarA regulation of the genes *cis* to these binding sites.

A. PCR-assisted binding site selection will be used to functionally select DNAs with SarA binding sites from a random pool of synthetic DNA fragments. The consensus binding site will be determined by computer-assisted alignment of functionally selected DNAs.
B. The consensus sequence for a SarA-binding site will be used in homology searches of existing *S. aureus* genomic databases. The search will be extended to include the entire *S. aureus* genome as it becomes available.
C. SarA regulatory control of the genes *cis* to putative SarA-binding sites will be tested by Northern blot analysis of wild-type strains and their corresponding *sar* mutants.

S. aureus is among the most persistent of all human pathogens. The continued emergence of antibiotic-resistant strains emphasizes the need to identify new therapeutic targets for the treatment of *S. aureus* infections. We believe the *sar* regulatory locus may be an appropriate target in that disruption of *sar*-mediated regulation has the potential to attenuate the bacterium to the point that it is more susceptible to clearance either by the normal host defense systems or existing antimicrobial agents. Accomplishing the specific aims outlined in this proposal will provide the foundation required to assess that possibility by establishing the correlation between *sar* transcription and SarA production and activity (Specific Aim #1), elucidating the mechanism

by which *sar* controls expression of a specific target gene (*cna*) (Specific Aim #2) and identifying additional SarA targets within the *S. aureus* genome (Specific Aim #3).