SUMMARY STATEMENT

PROGRAM CONTACT:
Martin Rogers
301-496-2544
jrogers@niaid.nih.gov

(Privileged Communication)

Release Date: **06/16/2010**

Application Number: **2 R01 AI064671-06**

Principal Investigator

STRIEPEN, BORIS PHD

Applicant Organization: UNIVERSITY OF GEORGIA (UGA)

Review Group: **PTHE**
Pathogenic Eukaryotes Study Section

Meeting Date: **06/03/2010**
Council: **OCT 2010**
Requested Start: **12/01/2010**

RFA/PA: **PA10-067**
PCC: **M91 B**

Project Title: **Biology of the apicomplexan plastid**

SRG Action: **Impact/Priority Score: 18 Percentile: 6**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
6	225,000	334,125
7	225,000	334,125
8	225,000	334,125
9	225,000	334,125
10	225,000	334,125
TOTAL	1,125,000	1,670,625

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

2R01AI064671-06 STRIEPEN, BORIS

SCIENTIFIC REVIEW OFFICER'S NOTES

RESUME AND SUMMARY OF DISCUSSION: This application seeks to continue using state-of-the-art cell biology, genetics and biochemical approaches to expand our knowledge of a unique and essential chloroplast-like organelle found in apicomplexan parasites, the apicoplast. The work focused on the characterization of the mechanisms involved in the apicoplast protein import machinery. The proposed studies are highly significant for the understanding of a parasite specific cellular structure and for the identification of novel drug targets. In addition, the work is of interest for comparative organelle biogenesis and protein transport mechanism. The previous project cycle generated results published in high impact journals as well as needed molecular tools and resources which are freely shared with community, thus promoting great advances in the field. The current research plan utilizes cutting edge transfection technology and a set of phenotypic assays developed in the investigator's laboratory. It is noted that the proposed aim 3 is beautifully designed. Minor concerns are expressed regarding Aim 2, focused on the apicoplast ubiquitination pathway, as it is unclear from the preliminary data provided if protein ubiquitination is important for protein import. Although there is some risk that protein ubiquitination might not be important for apicoplast protein import and overall the work is considered somewhat ambitious, there is confidence in the investigator's track record of accomplishment and believe that this application will continue producing valuable results and resources to advances the field of apicoplast biology. Overall, enthusiasm for this exciting application is high.

DESCRIPTION (provided by applicant): Apicomplexa are responsible for a number of important human diseases including malaria, toxoplasmosis, cryptosporidiosis and cyclosporidiosis. Management of these diseases rests heavily on chemotherapy but anti-parasitic drug treatment faces multiple challenges. These include poor overall potency, restriction to certain life-cycle stages, unwanted side effects, and rapidly emerging multiple drug resistance. A constant stream of new drugs and potential drug targets is required to stay abreast of the threat posed by these pathogens. One of the most promising sources of such parasite specific targets is the apicomplexan plastid or apicoplast. The apicoplast is unique to the parasite and its function is essential to parasite survival. This organelle is a holdover from a free-living photosynthetic past. The structure and biology of the apicoplast is remarkably complex as it is derived from the endosymbiotic marriage of two eukaryotes: a red alga and an auxotrophic protist. The goal of this application is to unravel the complexity of this biology in mechanistic detail and to identify future targets for intervention. Using Toxoplasma as a model organism we will conduct genetic, cell biological and biochemical approaches to characterize the function of two pathways that unfold in the outer compartments of the organelle and that we hypothesize are essential to the organelle and the parasites. We will complement this focused approach with a broader effort to define a comprehensive set of plastid proteins to continue to feed a pipeline of hypothesis-driven mechanistic experiments with strong candidate genes.

PUBLIC HEALTH RELEVANCE: *Toxoplasma gondii* is an important human pathogen that causes disease in the unborn fetus, young children and patients with a weakened immune system. We are a studying a unique cellular structure of the parasite that is related to the chloroplast of plants. A detailed understanding of the biology of this structure will lead us to new parasite specific interventions to treat and prevent disease.

CRITIQUE 1:

Significance: 2

Investigator(s): 1
Innovation: 2
Approach: 3
Environment: 2

Overall Impact:

Strengths

- The application seeks to identify the mechanisms of protein import into the apicoplast, an essential endosymbiotic organelle found in most apicomplexans. Defining the critical functions of this organelle is considered highly significant.
- Application of state-of-the-art genetic systems for regulated shutdown have been developed under the previous funding period and have been extremely important for testing function. This is further advanced by a novel system for combining improved strategies for KOs, with regulated gene expression.

Weaknesses

- There is some risk that protein ubiquitination will not be a major theme in apicoplast import and that bone fide substrates of this process will be difficult to identify. These studies would be more attractive if there were compelling evidence for the existence of this pathway and/or its importance in the apicoplast.

1. Significance:

Strengths

- The application seeks to identify the mechanisms of protein import into the apicoplast, an essential endosymbiotic organelle found in most apicomplexans.
- Apicomplexan parasites remain a highly significant cause of disease and treatments are often lacking or inadequate for many of these organisms.
- By elucidating apicoplast import pathways, it may be possible to design strategies to disrupt them, thereby providing an alternative therapeutic intervention. Although this is not the immediate focus here, it is a potential long term benefit of these studies.
- The project is also of interest for comparative biology of organelle biogenesis and protein transport mechanisms, a fundamental area of biology.

Weaknesses

- The investigator comments that the raison d'etre for the apicoplast is still unknown. It is not clear the present application will answer this key question, but rather simply catalogue with greater detail its functioning.

2. Investigator(s):

Strengths

- Strongly collaborative in contributing to a range of projects including biochemistry, metabolism, and genetics.
- Highly productive, and innovative and continues to produce outstanding work published in top journals.

- Excellent record of community service as well as running an outstanding research laboratory.

Weaknesses

- None noted

3. Innovation:

Strengths

- The use of split GFP to study protein import is highly innovative and has provided unique insights in this system, as well as being adaptable for many related systems,
- Likewise the development of quantifiable, biochemical assays for protein import has been extremely useful for defining the apicoplast function.
- Application of state-of-the-art genetic systems for regulated shutdown has been extremely important for testing function. This is further advanced by a novel system for combining improved strategies for KOs, with regulated expression.

Weaknesses

- The technological aspects and biological insights that are innovative could have been better highlighted by the investigator.

4. Approach:

Strengths

- The project is highly focused, very well rationalized and clearly explained.
- The focus on the ERAD system will provide better insight into how proteins cross the 4 membranes of the apicoplast. This is of fundamental interest and as well may identified key essential steps in the process.
- Experiments designed to test the ATPase function of Cdc48ap are well designed and take advantage of the best available methods.
- Approaches to studying protein ubiquitination are also well documented, highly feasible and should yield interesting information, provided this pathway is really robust and important.

Weaknesses

- There is some risk that protein ubiquitination will not be a major theme in apicoplast import and that one fide substrates of this reaction will be difficult to identify. These studies would be more attractive if there were compelling evidence for the existence of this pathway and/or its importance.

5. Environment:

Strengths

- Well equipped, newly constructed laboratory facilities are integrated into the same building with animal and imaging cores.
- Strong support for parasitology studies, excellent colleagues.
- Excellent university-wide core facilities.

Weaknesses

- None noted

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

Biohazards:

Acceptable

Renewal:

- Excellent progress has been made in the previous period with >6 major publications in top journals (PLoS, Curr Biol, PNAS, CHMicrobe, etc.). As well, important contributions have been made to collaborations and through authoring reviews.
- A variety of tools and resources have been developed and many of these have been adapted by others in the community.
- Findings on the import pathways in apicoplast have been cutting edge for parasitology and have generated cross-over interest from other fields.

Resource Sharing Plans:

Not Applicable (No Relevant Resources)

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 2
Investigator(s): 1
Innovation: 1
Approach: 2
Environment: 1

Overall Impact:

Strengths

- The investigator has been very productive in the first 5 years of this grant, generating a large body of work on the apicoplast as well as developing new methodologies for the Toxoplasma community. The planned studies in this renewal are ambitious, however they are likely feasible given the track record of the investigator.

- It is expected that these studies will give a complete picture of how the components of the apicoplast's protein import machinery function, whether the ubiquitin pathway exists in the apicoplast and what its function might be. These studies are significant for the Toxoplasma field, other Apicomplexans and beyond.
- Aim 3 is beautifully conceived and will give both the Striepen laboratory and the Toxoplasma community apicoplast proteins with which to work for years to come.

Weaknesses

- The data supporting the hypothesis to be tested in Aim 2 relies primarily on apicoplast localization of tagged E1 and E2. It is not clear from the application whether these are episomally expressed under a heterologous promoter. This needs to be clarified. If it is the case, more robust data would be needed upon which to base the hypothesis that the apicoplast utilizes the ubiquitin system either for some aspect of protein import or for another yet to be identified process.

1. Significance:

Strengths

- The data generated from the proposed experiments are highly significant. It is expected that they will complete the elucidation of the protein import machinery of the apicoplast, determine whether the ubiquitination pathway has some function in protein import into the apicoplast and lastly identify a new set of apicoplast proteins which will aid in determining the function of this unique organelle.

Weaknesses

- None

2. Investigator(s):

Strengths

- Dr. Striepen is an outstanding investigator who has been very productive in the initial period of this project. His collaboration with Dr. White for Aim 3 of this application is a clear strength and has enabled him to identify new apicoplast proteins for future work.

Weaknesses

- None

3. Innovation:

Strengths

- The proposed experiments are innovative, utilizing cutting edge transfection technology and an array of elegant phenotypic assays developed in the investigator's laboratory. Furthermore, via an innovative approach to mining the genome and transcriptome databases, the investigator has developed a unique system for identifying additional apicoplast proteins.

Weaknesses

- None

4. Approach:

Strengths

- The track record of the investigator in developing tools for genetically modifying Toxoplasma as well as the in-depth phenotypic analyses he has performed on published mutants, set the stage for this application. Thus a Cdc48 conditional mutant, already in hand, a variety of Cdc48 allelic mutants and a Ufd1 conditional mutant should not be a problem to generate and to obtain meaningful data from.
- The methodology used in Aim 3, to broaden the list of apicoplast proteins is very clever. In combination with a moderate-throughput method for tagging and localizing the candidate proteins that has been developed by the Striepen laboratory, this is powerful technology that should lead to the identification of new apicoplast proteins. Would be nice to see a Venn diagram of how the candidates generated via the phylogenetic analysis intersects with those generated from their transcription pattern.

Weaknesses

- The hypothesis upon which this aim is based relies heavily on the localization and western blot data of tagged E1 and E2. Stronger support in favor of pursuing this line of experimentation would be helpful.

5. Environment:

Strengths

- The environment at UGA is excellent and has all of the required facilities to undertake these studies.

Weaknesses

- None

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

Biohazards:

Not Applicable (No Biohazards)

Renewal:

- This is a competing renewal for an R01 on which the investigator has been very productive over the initial period. He has 14 publications directly related to this project as well as several other publications tangentially related to this work. The parent application focused on elucidating the mechanism(s) by which the apicoplast segregates, replicates, and divides and the investigator has answered these important biological questions as well as generated a significant amount of

data on apicoplast metabolism and protein import, some of which serves as the preliminary data for this renewal.

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 2
Environment: 1

Overall Impact:

Strengths

- The apicomplexan plastid (apicoplast) is an evolutionarily unique organelle that houses the targets of several drugs. The dissection of its functions has revealed both expected as well as novel activities.
- The present application expands on a highly productive project cycle that has contributed not only to our understanding of apicoplast biology but also stimulated the generation of molecular approaches and reagents in use by the field.
- The focus of the application of essential plastid functions demonstrates and exploits the utility of the conditional knock out strategy to functionally characterize previously intractable questions.
- The 2 primary pathways being investigated, those for apicoplast protein import and the role of ubiquitination as a regulatory component in plastid import and homeostasis are essential, novel and identified by the investigator in the course of the prior cycle.
- The third aim to actively seek out plastid genes by comparative bioinformatic approaches and systematic gene tagging and targeting will identify new aspects of parasite biology for in depth study beyond the scope of the application.
- Finally the strength of the investigator in the development of freely shared resources and technologies will continue to catalyze the field of apicoplast biology

Weaknesses

- There is some concern that the application as proposed is overly ambitious in its scope. There is not lack of confidence however that these aims will be addressed with the care and rigor the investigator is known for.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

- The investigator has extensive experience working with pathogenic organisms

Renewal:

- This is a competing renewal of a grant that has just completed its first cycle. The aims of the first cycle have been exceeded and resulted in several high impact papers. The work in the first cycle has established the paradigm for the systematic dissection of apicoplast biology. In addition, research technologies, reagents and tools developed in the fist cycle have accelerated the research endeavors of a broad crossection of Toxoplasma researchers. Finally, the completed work has crossed disciplines impacting diverse fields outside molecular parasitology.

Resource Sharing Plans:

Acceptable

- The investigator has consistently made resources generated in his laboratory freely available to the community.

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Given the scope of the work proposed, recommend an additional module be awarded.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

SCIENTIFIC REVIEW OFFICER'S NOTES: There were concerns that the requested budget might be insufficient to conduct the work as planned.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

MEETING ROSTER

Pathogenic Eukaryotes Study Section
Infectious Diseases and Microbiology Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW
PTHE
June 03, 2010 - June 04, 2010

CHAIRPERSON

SIBLEY, L. DAVID, PHD
PROFESSOR
DEPARTMENT OF MOLECULAR MICROBIOLOGY
WASHINGTON UNIVERSITY
ST LOUIS, MO 63110

MEMBERS

ADAMS, JOHN H, PHD
PROFESSOR
DEPARTMENT OF GLOBAL HEALTH
COLLEGE OF PUBLIC HEALTH
UNIVERSITY OF SOUTH FLORIDA
TAMPA, FL 33612

ALFONZO, JUAN D, PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY
OHIO STATE UNIVERSITY
COLUMBUS, OH 43210

ALMEIDA, IGOR C, DSC
ASSOCIATE PROFESSOR
DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF TEXAS AT EL PASO
EL PASO, TX 79968

ALSPAUGH, J ANDREW, MD
ASSOCIATE PROFESSOR
DEPARTMENT OF MEDICINE
DUKE UNIVERSITY MEDICAL CENTER
DURHAM, NC 27710

BELLOFATTO, VIVIAN , PHD *
PROFESSOR, VICE CHAIR FOR RESEARCH
DEPARTMENT OF MICROBIOLOGY
AND MOLECULAR GENETICS
UMDNJ- NEW JERSEY MEDICAL SCHOOL
NEWARK, NJ 07103

CORMACK, BRENDAN PATRICK, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF MOLECULAR BIOLOGY
AND GENETICS
SCHOOL OF MEDICINE
JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD 21205

DAVIS, RICHARD E, PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR
GENETICS
UNIVERSITY OF COLORADO
AURORA, CO 80045

DEEPE, GEORGE S JR, MD *
PROFESSOR
DIVISION OF INFECTIOUS DISEASES
COLLEGE OF MEDICINE
UNIVERSITY OF CINCINNATI
CINCINNATI, OH 45267

FRANCIS, MICHAEL M, PHD *
ASSISTANT PROFESSOR
UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL
WORCESTER, MA 01605

GALINSKI, MARY R, PHD *
PROFESSOR
DIVISION OF INFECTIOUS DISEASES
EMORY UNIVERSITY SCHOOL OF MEDICINE
EMORY UNIVERSITY
ATLANTA, GA 30329

GARG, NISHA JAIN, PHD
PROFESSOR
DEPARTMENT OF MICROBIOLOGY
AND IMMUNOLOGY
UNIVERSITY OF TEXAS MEDICAL BRANCH
GALVESTON, TX 77555

GAUSE, WILLIAM CLARK, PHD
PROFESSOR
DEPARTMENT OF MEDICINE
NEW JERSEY MEDICAL SCHOOL
UNIVERSITY OF MEDICINE
AND DENTISTRY OF NEW JERSEY
NEWARK, NJ 07101

GELLI, ANGELA , PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF PHARMACOLOGY
SCHOOL OF MEDICINE
GENOME AND BIOMEDICAL SCIENCES FACILITY
UNIVERSITY OF CALIFORNIA
DAVIS, CA 95616

GREENBERG, ROBERT M, PHD
RESEARCH ASSOCIATE PROFESSOR
DEPARTMENT OF PATHOBIOLOGY
SCHOOL OF VETERINARY MEDICINE
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PA 19104

KLEIN, BRUCE STEVEN, MD
PROFESSOR
DEPARTMENT OF PEDIATRICS,
MEDICAL MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF WISCONSIN-MADISON
MADISON, WI 53792

MELBY, PETER C, MD *
PROFESSOR
DEPARTMENT OF MEDICINE
DIVISION OF INFECTIOUS DISEASES
THE UNIVERSITY OF TEXAS
SAN ANTONIO, TX 78229

MITCHELL, AARON P, PHD
PROFESSOR
DEPARTMENT OF BIOLOGICAL SCIENCES
CARNEGIE MELLON UNIVERSITY
PITTSBURGH, PA 15213

MORENO, SILVIA N, PHD
PROFESSOR
DEPARTMENT OF CELLULAR BIOLOGY
CENTER FOR TROPICAL
AND EMERGING GLOBAL DISEASES
UNIVERSITY OF GEORGIA
ATHENS, GA 30602

NIERMAN, WILLIAM CHARLES, PHD
PROFESSOR
J.GRAIG VENTER INSTITUTE
ROCKVILLE, MD 20850

NOVER, MAIRI , PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF ORAL AND CRANIOFACIAL BIOLOGY
SCHOOL OF DENTISTRY
LOUISIANA STATE UNIVERSITY
NEW ORLEANS, LA 70119

PHILLIPS, MARGARET A, PHD
PROFESSOR
DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF TEXAS SOUTHWESTERN
DALLAS, TX 75390

SAMUELSON, JOHN C, MD, PHD
PROFESSOR
DEPARTMENT OF MOLECULAR
AND CELL BIOLOGY
GOLDMAN SCHOOL OF DENTAL MEDICINE
BOSTON UNIVERSITY
BOSTON, MA 02118

SHA, WILLIAM C, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF MOLECULAR
AND CELL BIOLOGY
UNIVERSITY OF CALIFORNIA, BERKELEY
BERKELEY, CA 94720

SINAI, ANTHONY P, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY
AND IMMUNOLOGY
COLLEGE OF MEDICINE
UNIVERSITY OF KENTUCKY
LEXINGTON, KY 40536

SINGH, UPINDER , MD
ASSISTANT PROFESSOR
DIVISION OF INFECTIOUS DISEASES
DEPARTMENT OF INTERNAL MEDICINE
AND MICROBIOLOGY
STANFORD UNIVERSITY
STANFORD, CA 94305

SINNIS, PHOTINI , MD *
ASSOCIATE PROFESSOR
DEPARTMENT OF MEDICAL PARASITOLOGY
SCHOOL OF MEDICINE
NEW YORK UNIVERSITY
NEW YORK, NY 10010

SMITH, JOSEPH D, PHD
ASSOCIATE MEMBER
SEATTLE BIOMEDICAL RESEARCH INSTITUTE
SEATTLE, WA 98109

VAIDYA, AKHIL B, PHD
PROFESSOR
DEPARTMENT OF MICROBIOLOGY
AND IMMUNOLOGY
COLLEGE OF MEDICINE
DREXEL UNIVERSITY
PHILADELPHIA, PA 19129

WILLIAMS, NOREEN , PHD *
PROFESSOR
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF BUFFALO, SCHOOL OF MEDICINE
BUFFALO, NY 14214

SCIENTIFIC REVIEW ADMINISTRATOR

BOUNDS, TERA , DVM, PHD
SCIENTIFIC REVIEW OFFICER
CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

GRANTS TECHNICAL ASSISTANT

WASHINGTON, KELLIE L
EXTRAMURAL SUPPORT ASSISTANT
CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.