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SUMMARY STATEMENT
(Privileged Communication)

PROGRAM CONTACT:
Rory Duncan Sr.
[redacted]

Release Date: **12/15/2013**

Application Number: **1 R03 AI111015-01**

Principal Investigator

RAPPLEYE, CHAD A PHD

Applicant Organization: Ohio State University

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

Meeting Date: **12/09/2013**
Council: **JAN 2014**
Requested Start: **04/01/2014**

RFA/PA: **PA11-262**
PCC: **M31**

Project Title: **Forward genetics-based discovery of Histoplasma virulence genes**

SRG Action: **Impact Score: 13**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
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
1	[redacted]	[redacted]
2	[redacted]	[redacted]
TOTAL	[redacted]	[redacted]

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.

1R03AI111015-01 RAPPLEYE, CHAD

RESUME AND SUMMARY OF DISCUSSION: This application seeks to identify *Histoplasma capsulatum* genes required for survival, replication and destruction of macrophages. The mechanisms involved in Histoplasma-macrophage interactions are not well known, thus the work is of high significance to expand our knowledge on the pathogenesis of this organism as well as other intracellular pathogens. The work benefits from the recent development and validation of a RFP-fluorescent Histoplasma strain and a transgenic lac-Z expressing macrophage cell line allowing quantitative monitoring of intracellular replication and macrophage destruction. The investigator has an excellent track record in the area and should be able to carry out the project successfully. A strong set of preliminary data are provided to support the aims of the application. The research plan is feasible and well-designed. Although a few minor weaknesses are identified; overall, there is high enthusiasm for this application and certainty that novel virulence genes will be identified with the proposed plan.

DESCRIPTION (provided by applicant): Current understanding of the molecular mechanisms that underly Histoplasma pathogenesis remains limited. Unlike opportunistic pathogens, the fungal pathogen *Histoplasma capsulatum* can cause disease even in immunocompetent hosts by parasitizing phagocytes of the host. Only a few virulence factors have been identified and characterized to date. In this proposal, we will use a forward genetics approach to discover the virulence factors that enable Histoplasma to subvert the defenses of the macrophage, Histoplasma's primary host cell. Random mutants of Histoplasma yeasts will be created using insertional mutagenesis. Mutants will be screened for decreased virulence in macrophages using a transgenic macrophage line and a Histoplasma strain that has been engineered with fluorescence to provide high-throughput screening capability. Mutants will be classified according to the stage at which Histoplasma pathogenesis is blocked by analysis of intramacrophage growth kinetics. The virulence genes represented by each attenuated mutant will be identified by mapping of the mutation. The final collection of virulence-defective mutants will be ranked according to the severity of their impairment, the classification of their pathogenesis defects, and the identity of the virulence gene identities. These rankings will be used to prioritize further characterization of the discovered virulence factors in future studies to define their roles in facilitating Histoplasma survival and growth in host macrophages.

PUBLIC HEALTH RELEVANCE: Histoplasmosis, a respiratory and systemic disease caused by infections with the fungal pathogen *Histoplasma capsulatum*, afflicts thousands each year in the United States regardless of the host's immune status. The mechanisms that enable Histoplasma to subvert immune defenses are poorly understood. This proposal will identify new virulence factors through a genetics approach to improve our understanding of Histoplasma pathogenesis. Identification of these processes essential to virulence will aid in the development of improved therapeutic options to treat histoplasmosis.

CRITIQUE 1:

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

Overall Impact: The application developed by this productive and talented investigator centers on a forward genetics approach to identify *Histoplasma capsulatum* genes that are involved in binding to macrophages and survival within these cells. The work is supported by strong preliminary data and accompanied by an excellent toolbox to perform the experiments in a high throughput manner. There is

little doubt that new data will be forthcoming. One weakness of this approach is that essential genes may be involved in these processes and will be missed because mutations will be lethal. For example, heat shock protein 60 is a ligand for CD11b and a mutation in the Histoplasma gene might not be discovered by this technology since it can be a lethal mutation. A second weakness is that a similar approach has been used by others to identify mutant phenotypes. For example, as the investigator points out a Cbp1 mutant has a strong phenotype as do several other genes. The investigator has not provided a powerful rationale for proceeding to add additional genes to this compendium.

1. Significance:

Strengths

- The identity of genes involved in promoting survival in macrophages is a fundamental query that begs to be answered.
- The findings may be applicable to a variety of fungal and perhaps non-fungal intracellular pathogens. In that regard, the data may be highly revealing.

Weaknesses

- Some of this work has been done by others using a similar technology. Therefore, it is not clear how this will add to existing progress.

2. Investigator(s):

Strengths

- The investigator is talented and highly productive. He has published a number of manuscripts that have altered our concepts about Histoplasma. He is capable of performing the experiments.

Weaknesses

- None noted.

3. Innovation:

Strengths

- The toolbox for investigating Histoplasma survival is technically innovative.

Weaknesses

- The agrobacterium transformation as pointed out by the investigator has been used by others.

4. Approach:

Strengths

- The technical approach is sound. It is direct and answers will likely be forthcoming.
- The questions posed are highly relevant to Histoplasma pathogenesis.
- The work is supported by excellent preliminary data including a publication.

Weaknesses

- Essential genes will be missed since mutations may be lethal.
- The accumulation of more genes than those we know is not well-justified. There are numerous candidates but little has been done to examine how they promote growth.

5. Environment:

Strengths

- The environment is outstanding.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Budget and Period of Support:

Recommend as Requested.

CRITIQUE 2:

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

Overall Impact: This R03 application proposes an innovative forward genetics screen to identify genes required for the survival of *H. capsulatum* in macrophages. The application is significant because very few such genes have been previously identified and it is likely that the insights that arise from studying such genes will have implications to other intracellular fungal pathogens. The investigator is outstanding and his laboratory is one of only a few in the world that have the expertise to accomplish these aims. The screen utilizes a novel screening strategy well-designed to its intended purpose and, hence, the application is innovative. There is ample preliminary data and pilot screening results to support the feasibility of the proposed screen. The Aims are well-thought out and the screening strategy is sound. This application has essentially no identifiable flaws.

1. Significance:

Strengths

- Only a small set of virulence genes for Histoplasma have been identified to date.
- Virulence factors identified by this screen are likely to have broader implications for other intracellular fungi.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- The investigator is productive and an expert in this area of investigation
- Few other laboratories are in a position to perform these studies.

Weaknesses

- None noted.

3. Innovation:

Strengths

- The application describes a novel screening strategy based on a clever combination of reporters

Weaknesses

- None noted.

4. Approach:

Strengths

- The preliminary data strongly support the feasibility of the proposed screen
- The screening plan is well-thought out and logical
- The hit analysis and prioritization is logical.

Weaknesses

- None identified

5. Environment:

Strengths

- The environment is strong.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Budget and Period of Support:

Recommend as Requested.

CRITIQUE 3:

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

Overall Impact: This is a succinct and well-written R03 application from a productive mid-level investigator that proposes to use a forward genetics approach to identify Histoplasma genes required for macrophage lysis and intracellular survival/growth. The investigator has developed an agrobacterium mutagenesis system, which inserts elements randomly into the genome and has developed a macrophage based screening system that can be performed in 96 well plates. Any target gene can be sequencing using a nested PCR approach. In aim 1, he proposes to generate a bank of 40,000 mutants with 2.5X genome coverage. For screening, the investigator has constructed lacZ expressing macrophages and RFP expressing Histoplasma to be able to simultaneously monitor *in vivo* growth and endpoint macrophage survival. Mutants can then be classified and prioritized based on the nature and degree of defect. The investigator has also shown proof of principle for the mutagenesis and screening techniques and already identified 21 mutants with these methods. One of these target genes has been sequenced and the resulting mutant is defective in virulence in an animal model of infection. The investigator has effectively calculated the feasibility of the studies and estimates 400 96 well plates will be needed for the screen, with 8-10 plates screened per week. The application is significant because there have been very few virulence factors identified in Histoplasma and mechanisms involved in survival within macrophages have not been well characterized. There is also a plan to translate results from this application into a future R01 application.

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:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html.
The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

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PTHE
December 09, 2013 - December 10, 2013

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