SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **03/21/2011**

Jukka Korpela

[redacted]

Application Number: **1 R21 AI096101-01**

Principal Investigator

STARNBACH, MICHAEL N PHD

Applicant Organization: HARVARD UNIVERSITY (MEDICAL SCHOOL)

Review Group: **ZRG1 IDM-A (80)**
Center for Scientific Review Special Emphasis Panel
RFA Panel: Topics in Bacterial Pathogenesis

Meeting Date: **03/07/2011** ***RFA/PA:*** **PA10-069**
Council: **MAY 2011** ***PCC:*** **M36**
Requested Start: **07/01/2011**

Project Title: **Alteration of host protein stability by Legionella**

SRG Action: **Impact/Priority Score: 10**
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		
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.

1R21AI096101-01 STARNBACH, MICHAEL

RESUME AND SUMMARY OF DISCUSSION: This application seeks to adapt a novel screening "Global Protein Stability" (GPS) strategy to identify host cell proteins whose stability is altered by *L. pneumophila* effectors. The work proposed is likely to have a significant impact by expanding our understanding of *L. pneumophila* effectors and extending the use of GPS to bacterial pathogenesis. Strengths of the application include the innovative use of the novel GPS strategy, compelling preliminary data, an investigator with a strong bacterial pathogenesis research track record, an excellent and appropriate set of collaborators, and a high degree of confidence that import results will emerge from these studies. The panel notes that the approach is limited to effectors that modify stability, that it may be difficult to sort out the details of instances where more than one effector is required to produce an affect, that changes in protein stability may involve a cascade of events that could prove difficult to deconvolute, and that the application is likely overly ambitious for the time and resources of an R21. None of these considerations (not viewed as concerns) had a dampening affect on the panel that maintained the highest level of enthusiasm for the investigator and work proposed.

DESCRIPTION (provided by applicant): Infection with the intracellular pathogen *Legionella pneumophila* can lead to a severe pneumonia known as Legionnaires' disease. *Legionella pneumophila* uses a specialized type IV secretion apparatus, also known as the Dot/Icm system, to secrete over 150 effector proteins directly into the host cell. The translocated bacterial effectors establish a vacuolar niche that supports replication of *L. pneumophila* in eukaryotic cells. While there is an extensive literature describing how several of these effectors alter host cell functions, the targets of most have remained elusive. A significant problem in linking a particular effector to a particular function is the redundant or overlapping activity of many effectors. This means that *L. pneumophila* mutant strains deficient in any one effector often have no appreciable phenotype, preventing the identification of their host targets. While it is well appreciated that many *L. pneumophila* effectors directly alter host proteins through functions such as E3 ubiquitin ligase activity, there have been few methods developed to monitor pathogen-induced changes in host protein stability on a large scale. Here we propose to apply a novel screening method called the "Global Protein Stability" (GPS) system to identify host cell proteins whose stability is altered by the secreted *L. pneumophila* effectors. Once we have identified host proteins that are stabilized or destabilized when a functional type IV secretion system is present, we will test whether reducing or increasing the prevalence of these proteins (attempting to reverse the effects of the *Legionella* effectors) impairs the capacity of *L. pneumophila* to replicate and survive within host cells. Once we identify which host proteins must be altered in order for *L. pneumophila* to replicate, we will take a targeted approach to identify which of the *L. pneumophila* effectors are causing these essential changes to host proteins. In addition, the GPS screen may also identify the targets of specific "families" of effectors that have remained elusive, such as the *L. pneumophila* E3 ubiquitin ligases. The directed approach we propose allows us to overcome the difficulties inherent in target identification, such as the redundancy of effectors, and identify the functions of effectors that have remained cryptic. Organism-induced alterations of the host are key to pathogenesis, yet it has previously not been possible to study alterations to individual host proteins at the scale the GPS system permits. The experiments described in this proposal allow, for the first time, dissection of how bacterial infection globally regulates host cell proteins and pathways beyond the transcriptional level.

PUBLIC HEALTH RELEVANCE: *Legionella pneumophila*, the causative agent of Legionnaires' disease replicates inside host cells. To manipulate the host cell and replicate intracellularly, the organism injects >150 of its proteins into host cells. The proposed research uses a large-scale approach to identify the targets of these injected bacterial proteins - identifying the host cell proteins that are destabilized or stabilized by the injected bacterial proteins. Once we identify which bacterial proteins are manipulating which host proteins, we can test methods to disrupt these interactions. This may lead to the development of new classes of antibiotics to treat bacterial infection.

CRITIQUE 1:

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

Overall Impact: This is an innovative application that will utilize a recently described array to screen for changes in protein stability using a Global Protein Stability screen that was originally described by another laboratory at HMS for the assessment of the global protein stability to E3 ligases. The investigator proposes to use the GPS system to globally monitor changes in protein stability upon *Legionella pneumophila* type IV infection. *Legionella* uses type IV secretion to inject > 150 effectors into host cells. The effectors support Legionella replication. Few of these effectors have a know mechanism of action due is some cases redundancy of effectors action. The investigator will utilize the GPS library to identify individual and families of proteins whose stability is modified by Legionella. This is a good extension of the original use of the library. Investigator is well experienced to conduct theses studies. The primary concern is the resolution of the analysis, which requires changes in protein stability, since effectors that elicit post-translational modifications may not resolve in this screen. The last aim, to screen the effects of the Legionella E3 ligases, assures the generation of some data and will establish the resolution of this assay. This aim could be utilized in feasibility experiments to setup system parameters. Nonetheless, there is a high degree of enthusiasm for this application, which represents a novel exploratory study. There is interest to determine if the GPS system can be adapted to study other aspects of bacterial pathogenesis that have been resistant to analysis due to redundant pathogenic effectors. The application is a good match for the exploratory goals of the R21.

1. Significance:

Strengths

- Legionella remains an important bacterial pathogen and model for type IV secretion.
- The presence of ~150 type effectors has limited the ability to dissect the molecular and cellular pathogenesis of this bacterium.
- Some of the approaches in the application address the redundancy issues with the analysis of legionella type IV effectors function.
- The analysis may allow a temporal assessment of the global host response to legionella infections.
- Long-term goal to target host proteins that are required for successful bacterial replication as novel classes of “host-based” antibiotics.

Weaknesses

- There are some concerns that proteins stability will not be reflected in the action of effectors. This is the most significant concern in the application that may ultimately limit the utility of resolving host targets and insight into how legionella pathogenesis (type IV) affects host metabolism
- Redundancy remains a concern with the characterization of the Legionella with/ and without type IV.
- Screen may not resolve effectors that yield a posttranslational modification to the host cell rather than affecting the stability. On the other hand, one can envision that the posttranslational modified protein may affect stability of downstream proteins within a specific pathway.

2. Investigator(s):

Strengths

- This is an experienced investigator, with expertise in bacterial pathogenesis and immunology.
- Investigator has an excellent publication record.

Weaknesses

- The investigator has not published on Legionella, but stated that recent work in his laboratory on the identification of cellular factors that constrain bacterial growth used Legionella as the model, indicating that the investigator has some experience working with this pathogen.

3. Innovation:

Strengths

- This is a highly innovative adaptation of the global protein stability vectors to assess bacterial pathogenesis. .

Weaknesses

- It is not clear that many of the legionella effectors will elicit a protein stability change in host protein expression

4. Approach:

Strengths

- The initial adaptation has identified many of the obvious controls and limitations within the gps system that will be analyzed to assess the significance of observed changes in stability to proteins in the array. Several approached were described for the validation of the observed host proteins that undergo changes in protein stability.
- GPS reported system seems well designed for stability analysis.
- Preliminary data suggest that there are subsets of host proteins that have undergone changes in the stability, both up and down, upon legionella infections.
- The GPS system is setup for validation of “hits” more stable or less stable host proteins under endogenous promoters and expression levels in cells.
- The last aim may provide the most direct application for the gps array, screening the host response to the bacterial effectors.
    - Transfecting the “GPS reporter” cell lines with individually with the genes encoding the 150 known and putative Dot/Icm-translocated effectors also known as the *L. pneumophila* “secretome”. Craig Roy at Yale University has agreed to provide us with their existing set of the 150 effectors, but no letter of agreement was provided.
    - Dissect how all E3 ligase domain containing effectors in L. pneumophila co-opt the eukaryotic ubiquitination machinery to target other host proteins for degradation

Weaknesses

- May be complicated to modulate the protein expression of the individual array components to establish protein concentration that yield detectable phenotypes.

5. Environment:

Strengths

- Outstanding collaborators will provide input towards the development of the project at quarterly intervals.
- Eventual generation of the entre genome of gfp-orfs reporter vectors is possible towards the end of the application period.
- Letter of support for Stephen Elledge states that advice and the libraries would be accessible to the investigator.

Weaknesses

- No letter from the Legionella biologist (Craig Roy).

Protections for Human Subjects:

Not Applicable (No Human Subjects)

CRITIQUE 2:

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

Overall Impact: This is a well-written and highly innovative application with the aim to understand how Legionella subverts the host cell to promote intracellular replication and infection. By the strategy to use a global screening of how Legionella infection can be coupled to altered stability of host cell proteins there is a high likelihood to circumvent the inherent difficulty with the large number of T4SS effectors with redundant function. There is a high probability to increase our understanding of how Legionella subverts the host cell to promote replication and to identify the host proteins that are important or even essential for replication of the pathogen. The final aim of the application to identify the effectors responsible for the effect on the individual host proteins is a more difficult task that may be difficult to complete within the framework of this R21 application but the strategy to employ an expression library of individual effectors is a valid strategy to achieve also this goal. The research environment is excellent.

1. Significance:

Strengths

- Legionella infections are a significant problem and the mechanism by which this pathogen subverts the host cell to promote infection is intriguing and a challenge to study

2. Investigator(s):

Strengths

- This is an experienced productive investigator working in a highly creative and excellent research environment

Weaknesses

- No major weakness

3. Innovation:

Strengths

- The strategy to look at global host protein proteins stability coupled to functional type IV secretion is highly innovative and a strategy with great potential to identify host proteins that are important for the survival/replication of Legionella and an nice way to get around the problem with large numbers of effector proteins with redundant function.

Weaknesses

- No significant weakness identified

4. Approach:

Strengths

- Innovative screening for host cells proteins with altered stability in response to functional T4SS
- Strategy to identify host proteins important for Legionella replication
- May also provide novel insight into basic cell biology
- Use of a library encoding all T4SS effectors
- Preliminary data support the feasibility of the work

Weaknesses

- All host cell proteins targeted may not have altered stability
- May prove difficult to identify the individual effectors responsible for the effect on a certain host protein as several effectors are likely to act in concert in targeting the host protein or signaling pathway

5. Environment:

Strengths

- The research environment is outstanding

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Not Applicable (No Relevant Resources)

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

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

Overall Impact: *Legionella pneumophilia* injects by a type 4 secretion system into the host cell over 50 effector proteins upon infection, and it is not known which host proteins are stabilized, and which are degraded upon infection. The applicant proposes to use the global stability system developed by Elledge and co-workers to identify which host proteins stabilized, and those that are destabilized upon *L. pneumophilia* infection. Once identified, the role specific individual proteins on infection will be assessed by either artificially stabilizing that protein by overproducing it, or inhibiting its synthesis using RNAi. In general, the proposed experiments are at the cutting edge in understanding how bacteria alter infected cells. It has high impact, and it is likely to be an important breakthrough for understanding microbial pathogenesis, and possibly new means for treatment.

1. Significance:

Strengths

- The proposal is highly significant, as it is among the first to attempt to look at host protein stability upon infection using a global approach. It could lead to novel approaches in treating specific diseases.

Weaknesses

- None apparent.

2. Investigator(s):

Strengths

- The applicant is full professor in the Department of Microbiology and Medical Genetics at Harvard University. He received his Ph.D. from Stanford University, and did postdoctoral work at the University of Washington. He has published on Legionella and other intracellular

pathogens, especially Chlamydia. He will be assisted by postdoctoral fellow Catarina Nogueria who has broad experience with Legionella.

Weaknesses

- None apparent.

3. Innovation:

Strengths

- The use of the global protein stability approach is highly innovative in studying microbial pathogenesis.

Weaknesses

- None

4. Approach:

Strengths

- Using the global protein stability approach is a very innovative methodology to look at protein stability on infection.
- The preliminary results presented indicate that the applicant will be successful in identifying specific proteins whose stability is altered on infection.

Weaknesses

- The figure on page 24 is confusing. In the text it states that the blue line represents the wild type, and the red represents the delta dot mutant, whereas in the legend it is reversed.
- Aim 2 is quite ambitious, and there may not be enough time to do all the experiments as proposed. The applicant is aware of this, as for example, he states if time permits he will carry out specific experiments as designed.

5. Environment:

Strengths

- The environment is excellent. The collaboration with others at that institution appears to be instrumental in the development of this proposal.

Weakness

- None apparent.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Not Applicable (No Relevant Resources)

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

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. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

Center for Scientific Review Special Emphasis Panel
CENTER FOR SCIENTIFIC REVIEW
RFA Panel: Topics in Bacterial Pathogenesis
ZRG1 IDM-A (80) S
March 07, 2011 - March 08, 2011

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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.