SUMMARY STATEMENT
(Privileged Communication)

PROGRAM CONTACT:
Kentner Singleton
240-669-5499
kentner.singleton@nih.gov

Release Date: **12/01/2022**
Revised Date:

Application Number: **1 R01 AI176639-01**

Principal Investigator

TROEMEL, EMILY R

Applicant Organization: UNIVERSITY OF CALIFORNIA, SAN DIEGO

Review Group: **ZRG1 IIDA-M (90)**
Center for Scientific Review Special Emphasis Panel
Innate Immunity and Inflammatory Responses

Meeting Date: **11/02/2022**
Council: **JAN 2023**
Requested Start: **01/01/2023**

RFA/PA: **PA20-185**
PCC: **I2A**

Project Title: **Innate immunity against viral infection in intestinal epithelial cells of C. elegans**

SRG Action: **Impact Score:10 Percentile:1 #**
Next Steps: **Visit https://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	250,000	386,374
2	250,000	386,374
3	250,000	386,374
4	250,000	386,374
5	250,000	386,374
TOTAL	1,250,000	1,931,871

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.

1R01AI176639-01 Troemel, Emily

RESUME AND SUMMARY OF DISCUSSION: This application proposes to identify the mechanisms of antiviral signaling and RNA immune sensing pathway in *Candida elegans* based upon previous research work. This proposal is highly significant as it seeks to advance our understanding of the innate immune response through the identification of an inducible anti-viral and anti-fungal response in *C. elegans*. Additional major strengths of the application include a well-established team of investigators with expertise in RNA sequencing and intracellular pathogen response pathway. The research approach includes comprehensive discussion of the rigor of prior research, technical innovation in the development of unique tools to explore the intracellular pathogen response pathway. There is extensive and compelling preliminary data supporting the feasibility of the work, adequate description of the potential pitfalls and alternative approaches. Overall, the review panel expressed a high level of enthusiasm for this strong application.

DESCRIPTION (provided by applicant): RNA viruses have had an immense impact on human health. SARS-CoV-2 is only the most recent of many RNA viral zoonoses, and, even disregarding pandemics, the health burden of endemic RNA viruses, particularly in vulnerable populations, is substantial. Epithelial cells, abundant and exposed at mucosal surfaces, are often the first to be infected by RNA viruses, and are therefore often the first cell type to detect and respond to viral infection. However, unlike circulating immune cells, their in vivo behaviors cannot be measured from blood draws, and their behavior ex vivo may poorly correlate with in vivo dynamics. Our long-term goal is to understand how epithelial cells coordinate anti-viral responses in a whole-animal setting. Our previous work demonstrated that the RIG-I-like receptor (RLR) DRH-1 in the nematode C. elegans activates an anti-viral transcriptional response in intestinal epithelial cells that we named the Intracellular Pathogen Response (IPR), which protects against infections by viruses and other intracellular pathogens. We found that DRH-1 responds to infection with Orsay virus–a single-stranded, positive-sense RNA virus that naturally infects C. elegans intestinal epithelial cells. The objective of this proposal is to determine where and how DRH-1 triggers resistance to Orsay virus infection, and investigate whether in C. elegans, which lacks identified homologs of interferons, there is a role for bystander cells in mounting an immune response. The central hypothesis is that upon Orsay virus infection, DRH-1 in intestinal epithelial cells detects viral replication and induces the IPR, signaling to neighboring cells through an as-yet undescribed pathway. The rationale is based on our genetic analysis of DRH-1 and its role in anti-viral responses, and our visualization of IPR gene expression and DRH-1 localization dynamics in the context of infection. Our work is innovative because we are pursuing the IPR, which shares similarity with the type-I interferon (IFN-I) response in humans, but excitingly, appears to signal through novel factors, as homologs of MAVS, IRF3, NFkB, TNF-alpha and IFN-I itself are absent from the C. elegans genome. We will test our hypothesis with three specific aims: Aim 1) Where and how does DRH-1/RLR promote anti-viral defense in C. elegans? Aim 2) What signaling pathway is activated downstream of DRH-1/RLR in C. elegans? Aim 3) Which host cells mount an anti-viral immune response in C. elegans? The expected outcomes are to establish the signaling cascade used by DRH-1/RLR to trigger the protective IPR immune response in intestinal epithelial cells of C. elegans, and to identify the components of a systemic defense system. The proposed research is significant, because it could lead to new treatments for infections by RNA viruses, as well as a better understanding of epithelial immune defense and inflammatory diseases.

PUBLIC HEALTH RELEVANCE: The proposed research is relevant to public health because it will characterize a novel form of immune defense against RNA virus infection in epithelial cells. By describing how the RIG-I-like receptor DRH-1 activates anti-viral defense in the nematode C. elegans, we may uncover novel forms of cell-intrinsic as well as systemic immune responses driven by epithelial

cells. Thus, the proposed research is relevant to the NIH's mission to seek fundamental knowledge about living systems in order to reduce the burden of disease.

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

Overall Impact: This new proposal from an outstanding investigator seeks to determine the mechanisms of antiviral signaling in C elegans. The project is based on the PIs recent identification of the Intracellular Pathogen Response (IPR) and the sensor Drh1 that mediates activation of the antiviral IPR during infection with Orsay virus, the only known viral pathogen of C elegans. The work will characterize Drh1 localization in response to virus infection, the signaling pathway that links Drh1 to induction of IPR genes, and the cell biology of responses in infected versus uninfected cells. Strengths include the well-suited background and expertise of the PI, the unique and powerful tools developed to address the aims, the high potential for new insights, and the strong relevance of comparative immunity studies to provide insight into the evolutionary origins of innate antiviral immunity. Minor weaknesses include the somewhat open-ended nature of the screens and mass spec approaches as described, but these are mitigated by outstanding collaborators and viable alternative approaches. Overall, there is extremely high enthusiasm for this outstanding grant application.

1. Significance:

Strengths

- The identification of an inducible anti-viral and anti-fungal response in C. elegans, along with an antiviral sensor (Drh1) is of high significance in furthering our understanding of innate immunity across evolutionary time.

Weaknesses

- None noted

2. Investigator(s):

Strengths

- Exceptional investigator who discovered the IPR and the role of Drh1 in the antiviral arm of the IPR.
- Excellent collaborators who provide complementary expertise for some of the more technically challenging experiments

Weaknesses

- None noted

3. Innovation:

Strengths

- The discovery of the IPR and its antiviral sensor, together with the unique tools developed to explore this pathway, are highly innovative

- The identification of domains in the N terminus of Drh1 that closely resemble CARDs in structure is novel, together with the fact that the N terminus of Drh1 alone can signal potent activation of the IPR. This holds promise to determine evolutionary roots of RLR signaling, with important implications for human antiviral defense

Weaknesses

- None noted

4. Approach:

Strengths

- Exceptionally well written and clear experimental plan
- Well-developed tools and approaches with which the PI (and collaborators) have extensive experience
- Complementary aims that will provide new insights into key players, signaling mechanisms, and cell biology of the C elegans antiviral response
- Careful attention to potential pitfalls and alternative approaches with viable back up plans

Weaknesses

- Some of the screens and mass spec approaches are open ended, which may not yield new insights. However, this weakness is viewed as minor given the power of the techniques and the well documented alternative approaches
- For the tagging of endogenous Drh1: since the N terminus resembles the 2xCARD of the mammalian RLRs, and this domain is known to oligomerize with MAVS, would it be worth also making a C terminal wrmScarlet fusion that may have less chance of interfering with function?

5. Environment:

Strengths

- The research environment is ideal for this project

Weaknesses

- None noted

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

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

Overall Impact: This is a new R01 application from Emily Troemel from the Department of Cell and Developmental Biology at UC San Diego. The PI has a very strong record in working with C. elegans and has enlisted Alistair Russel for expertise in single cell sequencing and analysis. She also has letters from three other investigators who have committed to sharing reagents for expanding studies into viral infection of C. elegans, sharing data from related screens, and providing key support for separating C Elegans into single cells. The Proposal is focused studying the intracellular pathogen response, first identified in the PI's lab. They identified the Dicer-related Helicase, a homolog of RIG-I-like receptors (RLRs) in mammals, as one of the regulators of this response and propose to determine how the DRH-1 controls antiviral immunity in the absence of mammalian homologs that act downstream of the RLRs. DHR-1 control of the response to Orsay virus will be studied in 3 specific aims, one to determine where DHR-1 is expressed and acts a second to identify the pathway that's activated in response to DRH-1 activation and a third, to determine which cells participate in the antiviral response. The PI's experience with this system is very evident in the carefully laid out plans and in the many alternative strategies that are laid out throughout this application. The main shortcoming I found was that the final "potential outcomes, pitfalls and future directions" section didn't tie things together very well, suggest bigger picture interpretations or suggest future directions.

1. Significance:

Strengths

- C elegans provides a very tractable, defined and well-studied system in which to explore biologic functions in a simple organism.
- The PI's lab has identified a sensing and signaling system in C elegans that is very similar to the RIG-1 and MDA5 system in mammals that senses aberrant cytoplasmic RNA, including the C elegans pathogen Orsay virus. They have also begun to define the downstream response. This positions them uniquely to research how this defense works and how it can be applied to mammalian models.
- This system seems to require interpretation before the findings can be applied to the mammalian system. Its simple nature and differences from the mammalian system however serve to eliminate preconceived biases and may well reveal new information with respect to how the mammalian immune system works or evolved.

Weaknesses

- None noted

2. Investigator(s):

Strengths

- Emily Troemel long history in C elegans research starting as a graduate student. Excellent publication record
- Alistair Russell co-investigator, expertise in single cell RNAseq analysis.

Weaknesses

- None noted

3. Innovation:

Strengths

- Application of the relatively simple and very tractable C elegans system to characterize what happens downstream of the RLR homolog DHR-1 in response to RNA virus infection.
- Application of a newly developed single-cell sequencing methodology to look at downstream signaling and to identify possible effectors.
- scRNA seq is as yet uncommon in C elegans work and thus likely to break new ground. The only caveat is the possible barrier of completely disrupting the worms into single cells, but the PI has enlisted the help of Dr. Kenyon at Calico who has had success with this.
- Ability to monitor the response to infection in every cell of a whole animal is unique.

Weaknesses

- None noted

4. Approach:

Strengths

- Very logical progression of experiments
- Solid redundancy to confirm and/or establish findings
- Potentially difficult experiments covered by collaborations or co-investigator.

Weaknesses

- The final “potential outcomes, pitfalls and future directions” section didn’t tie things together very well, suggest bigger picture interpretations or suggest future directions.

5. Environment:

Strengths

- Excellent resources both intellectual environment and material resources.

Weaknesses

- None noted

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

- included but Orsay virus is BSL1

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

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

Overall Impact: This is a new R01 application seeking to explore a yet-to-be defined RNA immune sensing pathway in C. elegans. Significance is high as evolutionary analysis of antiviral pathways in non-mammalian organisms often provide important insights. Rigor of prior research is strong as the investigator discovered the novel function of RIG-I homolog DCR-1 in inducing a transcriptional response they named Intracellular Pathogen Response (IPR). Investigator team is excellent with many years' experiences in this area of research including foundational work on the IPR pathway. Published and prelim data clearly established a general framework of a new pathway in C. elegans that senses Orsay RNA virus by DRH-1, then activates ZIP-1 and the IPR pathway. Three aims in approach covers key aspects of this pathway in a logical fashion, including highly mechanistic experimental design to uncover components of the pathway, cell and tissue specificity. Minor concerns include several approaches are exploratory, including genetic and proteomic screens to identify components of this new pathway, although the C. elegans model system should be amendable to these approaches. Also, IPR could have systemic effects, and Aim 3 should include genetic linage-tracing tools to better defined the origin of IPR signal. Overall, this application addresses fundamental immunobiology in a model organism with clear structure and experimental tools that should yield exciting results.

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 R01 AI176639-01; PI Name: Troemel, Emily R

Ad hoc or special section application percentiled against "Total CSR" base.

NIH has modified its policy regarding the receipt of resubmissions (amended applications).See Guide Notice NOT-OD-18-197 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.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

Center for Scientific Review Special Emphasis Panel
CENTER FOR SCIENTIFIC REVIEW
Innate Immunity and Inflammatory Responses
ZRG1 IIDA-M (90)
11/02/2022

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-22-044 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-22-044.html, including removal of the application from immediate review.

CHAIRPERSON(S)

SILVERMAN, NEAL, PHD
PROFESSOR
DEPARTMENT OF MEDICINE
UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL
WORCESTER, MA 01605

ACTING CHAIR

ELLIOTT, MICHAEL RUSTY, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY,
AND CANCER BIOLOGY
UNIVERSITY OF VIRGINIA
CHARLOTTESVILLE, VA 22908

MEMBERS

AYALA, ALFRED, PHD
PROFESSOR
DEPARTMENT OF SURGERY
RHODE ISLAND HOSPITAL
BROWN UNIVERSITY
PROVIDENCE, RI 02903

BAGAITKAR, JUHI, PHD
ASSOCIATE PROFESSOR
CENTER FOR MICROBIAL PATHOGENESIS
ABIGAIL WEXNER RESEARCH INSTITUTE
NATIONWIDE CHILDREN'S HOSPITAL
THE OHIO STATE UNIVERSITY COLLEGE OF MEDICINE
COLUMBUS, OH 43205

BARNES, BETSY J, PHD
PROFESSOR
INSTITUTE OF MOLECULAR MEDICINE
FEINSTEIN INSTITUTES FOR MEDICAL RESEARCH
MANHASSET, NY 11030

BEAULIEU, AIMEE MELISSA, PHD
ASSISTANT PROFESSOR
DEPARTMENT OF MICROBIOLOGY , BIOCHEMISTRY AND MOLECULAR GENETICS
CENTER FOR IMMUNITY AND INFLAMMATION
RUTGERS NEW JERSEY MEDICAL SCHOOL
NEWARK, NJ 07101

DE NORONHA, CARLOS M, SCD
ASSOCIATE PROFESSOR
CENTER FOR IMMUNOLOGY AND MICROBIAL DISEASE
ALBANY MEDICAL COLLEGE
ALBANY, NY 12208

DORFLEUTNER, ANDREA, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF ACADEMIC PATHOLOGY & DEPARTMENT OF
BIOMEDICAL SCIENCES
CEDARS SINAI
LOS ANGELES, CA 90048

FOXMAN, ELLEN FLESCHER, MD, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF LABORATORY MEDICINE
YALE SCHOOL OF MEDICINE
NEW HAVEN, CT 06511

LI, YAN CHUN, PHD
ASSOCIATE PROFESSOR
DIVISION OF BIOLOGICAL SCIENCES
DEPARTMENT OF MEDICINE
KNAPP CENTER FOR BIOMEDICAL DISCOVERY
UNIVERSITY OF CHICAGO
CHICAGO, IL 60637

QIN, XUEBIN, MD, PHD
PROFESSOR
DIVISION OF COMPARATIVE PATHOLOGY
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
TULANE NATIONAL PRIMATE RESEARCH CENTER
TULANE UNIVERSITY SCHOOL OF MEDICINE
COVINGTON, LA 70433

SANCHEZ-LOPEZ, ELSA, PHD
ASSISTANT PROFESSOR
DEPARTMENT OF ORTHOPAEDIC SURGERY
UNIVERSITY OF CALIFORNIA SAN DIEGO
LA JOLLA, CA 92093

STETSON, DANIEL B, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF IMMUNOLOGY
UNIVERSITY OF WASHINGTON
SEATTLE, WA 98109

YAN, NAN, PHD
PROFESSOR AND VICE CHAIR
DEPARTMENT OF IMMUNOLOGY
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER
DALLAS, TX 75390

SCIENTIFIC REVIEW OFFICER

DRAMMEH, BAKARY, PHD
SCIENTIFIC REVIEW OFFICER
CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

EXTRAMURAL SUPPORT ASSISTANT

OMANIDZE, EKA MURMANOVNA
EXTRAMURAL SUPPORT ASSISTANT
CENTER FOR SCIENTIFIC REVIEW
BETHESDA, MD 20892

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.