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See more samples online: http://www.niaid.nih.gov/researchfunding/grant/pages/appsamples.aspx

PI: **HOLM, GEOFFREY H**	Title: Mechanisms and Functional Consequences of Reovirus Innate Immune Activation	
Received: 06/24/2010	FOA: PA10-070	Council: 01/2011
Competition ID: ADOBE-FORMS-B	FOA Title: ACADEMIC RESEARCH ENHANCEMENT AWARD (PARENT R15)	
1 R15 AI094440-01	Dual:	Accession Number: 3310884
IPF: 1673901	Organization: COLGATE UNIVERSITY	
Former Number:	Department: Biology	
IRG/SRG: VIRB	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted]	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Geoffrey Holm	Colgate University	PD/PI
T.B.D. Research Technician	Colgate University	Technician
T.B.D. Undergraduate Research Assista	Colgate University	Undergraduate Student
John Parker	Cornell University	Other (Specify)-Other Significant Contributor

Appendices

Danthi 2010 preprint,Holm et al 201

This sample is a multi-page PDF document.

Continue scrolling to see the remainder of the application, navigate using the bookmarks in your PDF reader of choice, or skip to page 4 for the Table of Contents.

If you have any questions, contact deaweb@niaid.nih.gov .

OMB Number: 4040-0001
Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. a. Federal Identifier

b. Agency Routing Identifier

2. DATE SUBMITTED

Applicant Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** [redacted]

* Legal Name: Colgate University

Department: Biology

Division: Natural Sciences

* Street1: 13 Oak Drive

Street2:

* City: Hamilton

County / Parish: Madison

* State: NY: New York

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 13346-1338

Person to be contacted on matters involving this application

Prefix: Mr.

* First Name: Bruce

Middle Name:

* Last Name: Moseley

Suffix:

* Phone Number: [redacted]

Fax Number:

Email: [redacted]

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* [redacted]

7. * TYPE OF APPLICANT: O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Mechanisms and Functional Consequences of Reovirus Innate Immune Activation

12. PROPOSED PROJECT:

* Start Date: 04/01/2011

* Ending Date: 03/31/2014

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**

NY-023

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr.

* First Name: Geoffrey

Middle Name: Howard

* Last Name: Holm

Suffix:

Position/Title: Assitant Professor of Biology

* Organization Name: Colgate University

Department: Biology

Division: Natural Sciences and Math

* Street1: 13 Oak Drive

Street2:

* City: Hamilton

County / Parish: Madison

* State: NY: New York

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 13346-1338

* Phone Number: [redacted]

Fax Number: [redacted]

* Email: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	[redacted]
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	[redacted]
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Ms. * First Name: Helen Middle Name:

* Last Name: Kebabian Suffix: D

* Position/Title: Director, CFGR

* Organization: Colgate University

Department: Biology Division: Natural Sciences

* Street1: 13 Oak Drive

Street2:

* City: Hamilton County / Parish: Madison

* State: NY: New York Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 13346-1338

* Phone Number: [redacted] Fax Number:

* Email: [redacted]

*** Signature of Authorized Representative**: Bruce Moseley

*** Date Signed**: 06/24/2010

20. Pre-application Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific
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Appendix

Number of Attachments in Appendix: 2

OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tr bal government, academia, or other type of organization.

Organization Name: Colgate University

DUNS Number: [redacted]

* Street1: 13 Oak Drive

Street2:

* City: Hamilton

County: Madison

* State: NY: New York

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 13346-1338

* Project/ Performance Site Congressional District: NY-023

Project/Performance Site Location 1

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tr bal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City:

County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code:

* Project/ Performance Site Congressional District:

Additional Location(s) Add Attachment Delete Attachment View Attachment

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract 1234-Holm project summary.pdf Add Attachment Delete Attachment View Attachment

8. * Project Narrative 1235-Holm project narrative.pdf Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited 1236-BIBLIOGRAPHY AND REFERENCES CITE Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources 1237-Holm Resources.pdf Add Attachment Delete Attachment View Attachment

11. Equipment 1238-Holm Major Equipment.pdf Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

PROJECT SUMMARY

Diseases caused by reovirus in mammalian hosts are strain-specific: some type 1 viruses are associated with myocarditis, whereas type 3 viruses cause lethal encephalitis. The molecular mechanisms by which genetically similar reovirus strains cause these disparate diseases are not fully understood. However, pathologic hallmarks of these diseases are recapitulated in cell-culture models of reovirus infection. Encephalitis induced by type 3 reovirus correlates with the capacity of the strain to induce apoptosis both *in vivo* and in cell culture, whereas reovirus-induced myocarditis is associated with the limited capacity of the strain to induce type 1 interferons (IFNs), a family of related antiviral cytokines. Both IFN induction and apoptosis following reovirus infection are linked to innate immune responses dependent on transcription factors IRF-3 and NFκB. The central hypothesis of the proposed research is that prototype type 1 strain Lang (T1L) encodes an antagonist of innate immune response activation that alters regulation of cellular transcriptional networks that function to establish an antiviral state or induce apoptosis.

Three integrated specific aims are proposed to test this hypothesis. In Specific Aim 1, mechanisms for reovirus strain-specific differences in innate immune activation will be defined. The role of capsid stability in the cytoplasmic exposure of genomic dsRNA, a known inducer of innate immune responses, will be assessed using viruses with known capsid-destabilizing mutations. The genetic basis for these differences will be assessed using single-gene reovirus reassortants generated by reverse genetics. In Specific Aim 2, cellular transcriptional networks responsible for a) apoptosis and b) antiviral signaling will be defined by microarray analysis, using virus reassortants incapable of activating one or both pathways. Specific Aim 3 will facilitate the other two aims and enhance the undergraduate research program at Colgate University by introducing a laboratory component to an elective Virology course, which will engage students in a semester-long project to generate and characterize reovirus reassortants, and by supporting a senior-level research course in which students conduct the independent research projects outlined in Specific Aims 1 and 2.

Broadly, this research will enhance understanding of how genetically similar viruses can cause disparate diseases through manipulation of innate immune pathways. The proposed experiments also will provide insight into two long-standing questions in the dsRNA virus field: mechanisms of reovirus-induced apoptosis and mechanisms by which reovirus engages and counteracts cellular antiviral responses. As other pathogenic microbes employ similar strategies, this research program will uncover conserved mechanisms of infectious disease pathogenesis.

PROJECT NARRATIVE

Genetically similar viruses can cause widely disparate diseases through manipulation of innate immune pathways. This project uses mammalian reovirus, a powerful experimental model for studies of virus pathogenesis, to identify mechanisms for strain-specific differences in cellular responses to infection that influence organ-specific disease. As other pathogenic microbes employ similar strategies, this research program will uncover conserved mechanisms of infectious disease pathogenesis.

RESOURCES

Laboratory:
The PI has three well-equipped laboratory rooms in Olin Hall. One lab (~180 sq. ft.) is attached to the PI's office and contains lab benches and a sink. This lab is primarily used for electrophoresis and for media and solutions preparation. The main lab (~350 sq. ft.) is directly across the hall from the PI's office/lab and provides individual bench space for six researchers, as well as significant additional bench space for molecular biology. This lab contains a sink and chemical fume hood. An additional room (~190 sq. ft.) is accessed via a short corridor from the main lab. It contains two vertical laminar flow hoods (one dedicated for clean tissue culture and one dedicated for virus work), and additional bench space. Both of these laboratories are BSL2 compliant. Shared laboratory space in Olin Hall includes a microscopy suite, a 4°C cold room, and a 37°C warm room.

Computers:
The PI uses a MacBook4 laptop computer and has network access to the University servers and library, and shared departmental HP LaserJet 4100 series printer and Xerox Phaser 8550DT color printer. A shared departmental poster printer is also available. Software includes Microsoft Office, Adobe Photoshop, Adobe Acrobat, GraphPad Prism, and internet browsers. The laboratory contains an HP dc7900 desktop computer for controlling molecular devices and for student use.

Office:
The PI has a well-furnished office (~150 sq. ft.) within Olin Hall, which houses the computer and relevant files, personal library, etc. Departmental and Divisional Administrative Assistants provide administrative support.

Other (Environment):
Institutional Support: Colgate University is a highly selective private, liberal arts institution with ~2800 undergraduate students (www.colgate.edu). Colgate is committed to providing its student population with an intensive, high-quality education, and to build on this foundation to foster academic excellence. A critical component of this commitment is to enhance student-faculty research, and therefore Colgate continues to build on its signature programs in undergraduate scholarship. For science faculty, Colgate provides generous start-up funds and research space that meet the needs of new faculty to establish an active research program. The Colgate Research Council awards competitive, in-house research grants to faculty on an annual basis, including support for student assistants and for publication costs. Additionally, the Picker Institute for Interdisciplinary Study in the Sciences and Mathematics also awards ~$200,000 annually to Colgate faculty and their collaborators on a competitive basis. The Dean of the Faculty and the Director of Natural Sciences and Mathematics provide funds annually for faculty to participate in conferences, and dispense discretionary funds to support unexpected research and teaching needs. Finally, a significant institutional investment that supports research in biology and biomedicine is the Colgate NIH Study Group. Colgate has a unique relationship with the NIH, wherein 12-16 students spend the fall semester in Bethesda/Washington D.C. and work in labs at the NIH, receiving two course credits. Many students receive summer internships prior to the semester, giving them at least 6 months in the lab. A Colgate faculty member, from the Departments of Biology or Chemistry or the Program in Neuroscience, oversees the Study Group, including coordinating placement of students into labs and teaching two additional courses for Colgate students while in Bethesda. The faculty member may also

work in an NIH lab to support his or her own research interests, when feasible. This program fosters excellence in scientific research at Colgate, as it facilitates student exposure to the breadth of research opportunities that are available, and provides training in advanced research techniques and the scientific process. Many students return to Colgate enthusiastic to continue their research experience, and therefore seek to work in the labs of Colgate faculty members. These students not only have the intellectual and technical skills to take on more sophisticated projects, but can also provide leadership in the lab to students with less experience. Four of my research students have had experience at the NIH prior to joining my lab, and three of them have received Departmental Honors for their projects in my lab.

Divisional Support: To support the scientific community at Colgate, the Division of Natural Sciences and Mathematics hosts a weekly science colloquium, highlighting student-faculty research and hosting regional and national speakers, and biweekly Interdisciplinary Lunches for faculty members to gather and share their research interests. The Division also awards research fellowships to ~ 80 science students every summer, allowing them to spend 8 - 10 weeks working with faculty members.

Departmental Support: The Department of Biology is comprised of 13 FTE, one lab instructor, five technical staff, and an administrative assistant. In recent years, an average of ~ 55 students graduated each year with a B.A. degree in the biological sciences (Biology, Molecular Biology, Environmental Biology, or Marine/Freshwater Science). The department is housed both in Olin Hall and the new Ho Science Center, and offers excellent facilities including dedicated teaching labs and individual research labs for all faculty members. Student research is a departmental priority, as participation in undergraduate research projects is required of Biology and Molecular Biology majors at Colgate. Students most often fulfill this requirement by taking a one-semester "research tutorial" course in which a group of 5-6 students each carry out independent projects with a single faculty member. Many students also seek additional research opportunities through independent study or summer research projects. This research is supported through course budgets (e.g., $[redacted] per Research Tutorial); summer research and independent study funds (~ $[redacted] per student); and dedicated endowed funds (~ $[redacted] per year, depending on need). Additionally, the Department of Biology supports students to attend conferences and report their research results, and several biology students receive university support to travel with other Colgate students to present their research at the Annual Conference of the Council for Undergraduate Research. To enhance and broaden the reach of our courses and research, the Department hosts a weekly seminar series, which features regional and national speakers and provides opportunities for students to present their research. Technical staff provides support for teaching laboratories, including the proposed Virology laboratory component, and for equipment and material maintenance.

Faculty with research labs located in contiguous space in Olin Hall to the Holm lab include: Dr. Nancy Pruitt (freeze/desiccation tolerance of gall flies); Dr. Kenneth Belanger (molecular dynamics of nuclear import/export in yeast); Dr. Jason Meyers (retinal development in zebrafish); Dr. Barbara Hoopes (molecular genetics of dog body size and behavior); Dr. Engda Hagos (chromosome stability in cancer); and Dr. Jun Yoshino (experimental mouse models of multiple sclerosis). These colleagues, who regularly teach in the Biol 212 course, form an informal, but vibrant, scientific and pedagogical working group. Interactions include planning sessions for courses and preparation of scientific proposals, shared journal clubs and poster sessions for research students, and a variety of departmental social events.

Support for Early Stage Investigators: Colgate is committed to the success of Early Stage Investigators. In addition to generous start-up packages, Colgate supports a one-semester (two course-load reduction) Junior Faculty Leave upon successful completion of a Third-Year Review. Coupled with the semester leave proposed in this application, I would therefore have a full year of 100% effort on this project. The University provides opportunities for further training in research and teaching practices through the Faculty Development Council, which provides funds to attend training programs and conferences. The Department has a formalized mentoring program for junior faculty through the introductory courses Biol 211 (Evolution, Ecology, and Diversity) and Biol 212 (Molecules, Cells, and Genes), which are team-taught and pair junior faculty members with senior faculty members. Informal mentoring is encouraged through a weekly seminar series, a shared faculty lunchroom, and the close proximity of faculty offices and research labs to each other.

The University also enables formal mentoring relationships with senior faculty members outside of the Department, to provide advice on adjusting to the University at large, including the Core curriculum, and for confidential discussions of topics that might not be appropriate for discussing with close peers within the department.

Extra-institutional Support: Since the Department does not have other members with expertise in Virology and Immunology, I have continued my relationship with Dr. John Parker, at Cornell University in Ithaca, NY (~70 miles from Colgate; see letter of support). Dr. Parker has substantial expertise in the reovirus field, and is similarly interested in cellular responses to reovirus infection. He has given a seminar at Colgate, in association with my Virology class and Research Tutorial, and has hosted my summer research students in his lab to broaden their experience in working with reovirus. He has also extended the offer for my students and me to attend the Cornell Program in Virology Seminar Series and Annual Retreat, as a means of increasing exposure of Colgate students to current research in the field of virology. Dr. Parker has also generously offered to provide technical support and assistance when necessary, and to facilitate access to instrumentation that is not available at Colgate, such as a fluorescence-activated cell sorter.

A variety of other resources are available for undergraduate research at primarily undergraduate institutions. One resource that is relevant for this proposal is the Genome Consortium for Active Teaching (GCAT), a network of faculty members with interests in genomics and bioinformatics (see letter of support from Dr. Malcolm Campbell). This resource provides low-cost microarray slides and scanning facilities for use in undergraduate research, as well as technical support for these experiments. I have experience with GCAT-provided microarrays, and will continue to utilize this resource.

In summary, a wide range of resources is available to help ensure the success of this project. Colgate has a strong reputation for producing students who are well prepared for, and committed to, continued education after obtaining their baccalaureate degrees. In the most recent decadal survey by Franklin and Marshall College of the number of doctoral degrees awarded in the Life Sciences to students from 250 private undergraduate institutions, the Colgate Department of Biology (along with Psychology in Life Sciences) ranked 19th. In addition, ~ 25% of Biology students continue their studies in the health sciences either directly after graduating or within the following two-to-three years. Therefore, the research program outlined here targets a broad collection of talented students with a history of continuing their education in graduate and professional schools, and will continue to strengthen the undergraduate research program at Colgate University.

MAJOR EQUIPMENT:

Major equipment in the PI's lab includes a Veritas microplate luminometer, Techne3000 thermal cycler, Fisher Isotemp water-jacketed CO_2 incubator, liquid nitrogen cell storage system, compound inverted light microscope, variable speed microfuge, analytic balance, pH meter, water filtration system, several gel rigs for agarose and polyacrylamide gene electrophoresis, 3 power supplies, 2 refrigerators, 2 -20º C freezers, water baths, hot plates, stirrers, etc.

Shared departmental resources include: an ABI 3130 4-capillary DNA sequencer; ABI Prism 7900-HT real time PCR machine; Zeiss LSM 710 confocal microscope; Nikon E400 (4) and E600 (1) epifluorescence light microscopes with DIC or phase contrast optics, automated image capture, and Spot-RT or CCD digital cameras; NanoDrop spectrophotometer; MJ Research thermocyclers (3); New Brunswick heated shaker incubators; So-Lo -80º C freezers (2); Sorvall L5-50 ultracentrifuge and associated rotors; Sorvall RT6000 refrigerated benchtop centrifuge; Sorvall RC-5B refrigerated superspeed centrifuges (2); BioRad gel documentation system; speed vac; and walk-in cold (4º C) and warm (37º C) rooms. All are available within Olin Hall.

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Geoffrey Middle Name: Howard
* Last Name: Holm Suffix:
Position/Title: Assitant Professor of Biology Department: Biology
Organization Name: Colgate University Division: Natural Sciences and Math
* Street1: 13 Oak Drive
Street2:
* City: Hamilton County/ Parish: Madison
* State: NY: New York Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 13346-1338
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: Ph.D.
Degree Year: 2004

***Attach Biographical Sketch** 1245-Holm BioSketch.pdf Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 1

Prefix: * First Name: T.B.D. Middle Name:
* Last Name: Research Technician Suffix:
Position/Title: Research Technician Department:
Organization Name: Colgate University Division: Natural Sciences and Math
* Street1: 13 Oak Drive
Street2:
* City: Hamilton County/ Parish: Madison
* State: NY: New York Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 13346-1338
* Phone Number: [redacted] Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Technician **Other Project Role Category:**

Degree Type: M.S.
Degree Year:

***Attach Biographical Sketch** 1246-Technician BioSketch.pdf Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: | * First Name: T.B.D. | Middle Name:
* Last Name: Undergraduate Research Assistant #1/2 | Suffix:
Position/Title: Undergraduate Research Assistant | Department: Biology
Organization Name: Colgate University | Division: Natural Sciences and Math
* Street1: 13 Oak Drive
Street2:
* City: Hamilton | County/ Parish:
* State: NY: New York | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 13346-1338
* Phone Number: [redacted] | Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Undergraduate Student | **Other Project Role Category:**

Degree Type:
Degree Year:

***Attach Biographical Sketch** 1247-Summer student #1 BioSke | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 3

Prefix: Dr. | * First Name: John | Middle Name:
* Last Name: Parker | Suffix:
Position/Title: Associate Professor of Virology | Department: Baker Inst. for Animal Health
Organization Name: Cornell University | Division: College of Veterinary Medicine
* Street1: Hungerford Hill Road
Street2:
* City: Ithaca | County/ Parish:
* State: NY: New York | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 14853-6401
* Phone Number: [redacted] | Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Other (Specify) | **Other Project Role Category:** Other Significant Contributor

Degree Type:
Degree Year:

***Attach Biographical Sketch** 1248-ParkerNIHBioS_Holm6-23.p | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

To ensure proper performance of this form; after adding 20 additional Senior/ Key Persons; please save your application, close the Adobe Reader, and reopen it.

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Holm, Geoffrey Howard	Assistant Professor of Biology, Colgate University
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Bates College, Lewiston, ME	B.S.	05/97	Biology
Harvard Medical School, Boston, MA	M.M.Sc.	05/99	Medical Science
Harvard University, Cambridge, MA	Ph.D.	09/04	Virology
Vanderbilt University Medical Center, Nashville, TN	Post-doc	06/08	Microbiology and Immunology

A. Personal Statement.

The proposed research investigates mechanisms and functional consequences of reovirus innate immune activation, while building a strong foundation of undergraduate virology research at Colgate University. My broad background training in virology, immunology, and cell and molecular biology, coupled with my experience as an undergraduate educator, will facilitate the success of the project.

My major focus, in both my graduate and post-doctoral training, has been on cellular responses to viral infection. As a postdoctoral fellow at Vanderbilt University, I received a Ruth L. Kirschstein Post-doctoral National Research Service Award (NRSA) to investigate the role of IRF-3 in cell death responses to reovirus infection. This research resulted in two primary author publications, in the *Journal of Biological Chemistry* and the *Journal of Virology*, and several co-authorships. This post-doctoral training forms the foundation for my independent research program. Specific to the proposed work, I have considerable experience with the propagation and manipulation of reovirus, and I contributed to the development of a reovirus reverse genetics system. I have practical experience with microarray techniques and analysis, and all of the cell and molecular techniques proposed here.

As an Early Stage Investigator, I have cultivated relationships with other local virologists, to continue to gain experience and training. I am also mentored by members of the Biology Department at Colgate University, who have had successful experiences with administration and implementation of R15 AREA grants. As the proposal is dependent upon undergraduate research, I have supervised 20 undergraduate student researchers since 2005 (see below), including 4 who have received Departmental Honors for their projects. I also have experience teaching Virology and Microbiology, and developing and implementing investigative laboratory components for Microbiology and for an introductory Cell and Molecular Biology course.

In summary, I believe my background and training has fully prepared me for execution of this proposal, which will firmly establish my independent undergraduate research program in innate immune responses to reovirus infection.

Supervised 20 undergraduate student researchers since 2005.

<u>Colgate undergraduate research students (2008 – present)</u>

Brian Peddie (2008-2010)	Danielle Mercado (2009)	Isaac Syrop (2009)	Tracie Lo (2009-2010)
Erin Soule (2008 – 2009)	Shaina Musco (2009)	Nedelina Teneva (2009)	Jonathan Knowlton (2010)
Adam Bailey (2009)	Gretchen Polinski (2009)	Carly Turro (2009)	Anand Komareth (2010)
Jennifer Bradley (2009)	Lawrence Schweitzer (2009)	Marcela Azevedo (2009-2010)	Yeva Rybak (2010)
Tracy Hosley (2009)			

B. Positions and Honors.

Professional Positions

2002, 2004 Senior Teaching Fellow, Harvard College, Cambridge, MA
2005-2008 Post-doctoral Fellow, Dept. of Pediatric Infectious Diseases, Vanderbilt University, Nashville, TN
2008 - Assistant Professor of Biology, Colgate University, Hamilton, NY

Awards and Honors

Ruth L. Kirschstein Post-doctoral National Research Service Award (NRSA), 2006-2008.
1st Prize, Animal and Clinical Models; Vanderbilt University Medical Center Poster Competition, 2008.
Harvard College Certificate of Distinction in Teaching, 2002

Other Experience and Professional Memberships

2009-2011 Member, Membership Review Committee, American Society for Virology

Ad hoc reviewer: Journal of Virology (2008-); Virology (2009-).

Professional Memberships:
American Society for Virology
American Society for Microbiology
American Association for the Advancement of Science
Sigma Xi
Phi Beta Kappa

C. Publications.

Most relevant to the current application: (* = provided in appendix).

1. O'Donnell, S.M., **Holm, G.H.,** Pierce, J.M., Tian, B., Watson, M.J., Chari, R.S., Ballard, D.W., Brasier, A.R., and T.S. Dermody. 2006. Identification of an NF-κB-dependent gene network in cells infected by mammalian reovirus. J. Virol. 80: 1077-1086. PMCID1346919.

2. **Holm, G.H.**, Zurney, J., Tumilasci, V., Leveille, S., Danthi, P., Hiscott, J., Sherry, B., and T.S. Dermody. 2007. Retinoic acid-inducible gene-I and interferon-beta promoter stimulator-1 augment proapoptotic responses following mammalian reovirus infection via interferon regulatory factor-3. J. Biol. Chem. 282: 21953-21961. NIHMS209064.

3. Kobayashi, T., Antar, A.A.*, Boehme, K.W.*, Danthi, P.*, Eby, E.A.*, Guglielmi, K.M.*, **Holm, G.H.***, Johnson, E.M.*, Maginnis, M.S.*, Naik, S.*, Skelton, W.B.*, Wetzel, J.D.*, Wilson, G.J.*, Chappell, J.D., and T.S. Dermody. 2007. A plasmid-based reverse genetics system for animal double-stranded RNA viruses. Cell Host Microbe. 1: 147-157. PMCID2034303.

4. Danthi, P., Kobayashi, T., **Holm, G.H.**, Hansberger, M.W., Abel, T.W., and T.S. Dermody. 2008. Reovirus apoptosis and virulence are regulated by host cell membrane penetration efficiency. J. Virol. 82: 161-172. PMCID2224352.

5. Zurney, J., Kobayashi, T., **Holm, G.H.,** Dermody, T.S., and B. Sherry. 2009. Reovirus mu2 protein inhibits interferon signaling through a novel mechanism involving nuclear accumulation of interferon regulatory factor 9. J. Virol. 83: 2178-2187. PMCID2643726.

6. [redacted]

7. *Danthi, P., Pruijssers, A.J., Berger, A.K., **Holm, G.H**., Zinkel, S.S., and T.S. Dermody. 2010. Bid regulates the pathogenesis of neurotropic reovirus. PLOS Pathogens. In press. PMC Journal – In Process.

Additional publications (in chronological order):

1. Gorry, P.R., Taylor, J., **Holm, G.H.,** Mehle A., Morgan, T., Cayabyab, M., Farzan, M., Wang, H., Bell, J.E., Kunstman, K.E., Moore, J.P., Wolinsky, S.M., and D. Gabuzda. 2002. Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate. J. Virol. 76: 6277-6292. PMCID136234.
2. Yang, X, Mahony E**, Holm, G.,** Kassa, A, and J. Sodroski. 2003. Role of the gp120 inner domain beta sandwich in the interaction between the human immunodeficiency virus envelope glycoprotein subunits. Virology. 313: 117-25.
3. **Holm, G.H.,** Zhang, C., Gorry, P.R., Peden, K.W., Schols, D., De Clercq, E., and D. Gabuzda. 2004. Apoptosis of bystander T cells induced by human immunodeficiency virus type 1 with increased envelope/receptor affinity and coreceptor binding site exposure. J. Virol. 78: 4541-4551. PMCID387714.
4. **Holm, G.H.,** and D. Gabuzda. 2005. Distinct mechanisms of $CD4^+$ and $CD8^+$ T cell activation and bystander apoptosis induced by human immunodeficiency virus type 1 virions. J. Virol. 79: 6299-6311. PMCID1091688.
5. Gorry, P.R., Dunfee, R.L., Mefford, M.E., Kunstman, K., Morgan, T., Moore, J.P., Mascola, J.R., Agopian, K., **Holm, G.H.,** Mehle, A., Taylor, J., Farzan, M., Wang, H., Ellery, P., Willey, S., Clapham, P., Wolinsky, S.M., Crowe, S.M., and D. Gabuzda. 2007. Changes in the V3 region of gp120 contribute to unusually broad coreceptor usage of an HIV-1 isolate from a CCR5 Delta32 heterozygote. Virology. 362: 163-178. PMCID1973138.
6. Guglielmi, K.M., Kirchner, E., **Holm, G.H.,** Stehle, T., and T.S. Dermody. 2007. Reovirus binding determinants in junctional adhesion molecule-A. J. Biol. Chem. 282: 17930-17940.

D. Research Support.

Current research support:

None.

Completed research support:

Ruth L. Kirschstein Post-doctoral National Research Service Award: "Innate Immune Responses in Reovirus Infection."
July 2006 – June 2008.
Determined the role of IPS-1 and IRF-3 in reovirus apoptosis and described the role of IRF-3 in reovirus pathogenesis *in vivo*.
Role: PI/Post-doctoral fellow

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Research Technician	Research Technician
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
??	B.A.	??	Biology
??	M.S.	??	Biology

Research and Professional Experience:

Dependent upon applicants. The person hired will have some advanced training or relevant experience in molecular or cell biology techniques.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Summer Undergraduate Student #1/2	Summer undergraduate research student
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Colgate University		??	Biology/Molecular Biology

Research and Professional Experience:

Dependent upon applicants. Student accepted will most likely have just completed his/her sophomore or junior year. Colgate students at this stage in their coursework have had an intensive lecture and lab course (Biol 212: Molecules, Cells, and Genes) providing an introduction to molecular and cell biology, and most will also have completed our lecture and lab course on advanced molecular biology and molecular techniques (Biol 321: Molecular Analysis). Many students will also have taken at least one other upper-level molecular biology elective; those applying for this position will likely have taken either Microbiology (Biol 333), Virology (Biol 373), or Immunology (Biol 374).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Parker, John S.	Associate Professor of Virology
eRA COMMONS USER NAME [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Glasgow University, Glasgow, Scotland, UK	D.V.M.	1983	Vet. Medicine & Surgery
Cornell University, Ithaca, New York	Ph.D.	1999	Virology
Cornell University, Ithaca, New York	Postdoctoral	1999-2000	Virology
Harvard Medical School, Boston, Massachusetts	Postdoctoral	2002-2003	Virology

A. Personal Statement.

My role in this NIH-AREA proposal is to provide support to the principal investigator, Dr. Geoff Holm. My laboratory studies the mechanisms by which mammalian reoviruses induce apoptosis in cells and the role of viral chaperones in reovirus replication. My labs work on reovirus-induced apoptosis is directly pertinent to the focus of this NIH-AREA grant as Dr. Holm is investigating the role of interferon-dependent and -independent transcription in reovirus-induced apoptosis. Reoviruses are in phase II/III trials as oncolytic agents for the treatment of a variety of human cancers; the virus kills the transformed cells by induction of apoptosis. Thus, Dr. Holm's proposal will provide critical data needed to understand how reoviruses kill cancer cells. In this proposal I will provide support by continuing to host Dr. Holm and his research students in my lab and provide access for his undergraduate students to our facilities and reagents as needed. I will also arrange for Dr. Holm and his students to attend our Cornell University Program in Virology Annual retreat and seminars in virology (normally 2 or 3 per semester). In addition, I will facilitate access to specialized equipment, not available at Colgate when needed. Finally, I can provide other technical support and assistance, when necessary. I have a background in molecular virology, with specific training and expertise in reovirus virology. As a postdoctoral fellow at Harvard Medical School, I carried out research that identified the reovirus μ1 protein as the primary determinant of virus-induced apoptosis. As a PI on externally funded and NIH grants, I have trained three graduate students and two post-doctoral associates and numerous undergraduate students. My lab regularly hosts undergraduate researchers from Cornell and we will be delighted to host undergraduate students from Dr. Holm's group as requested.

B. Positions and Honors.

Positions and Employment

1983-1985 Associate Veterinarian, Cockburn and Nadin Veterinary Surgery, Leicestershire, UK.
1985-1987 Veterinarian various small animal practices, Australia and UK.
1987-1993 Associate Veterinarian, Whitehorse Veterinary Hospital, Melbourne, Australia.
1989-1993 Veterinarian, Animal Emergency Center Pty Ltd., Melbourne, Australia.
1993-1994 Consultant Veterinarian, Feline Health Center, College of Veterinary Medicine, Cornell University, Ithaca, NY.
2002-2003 Instructor, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston MA.
2003-2009 Assistant Professor of Virology, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY.
2009-present Associate Professor of Virology, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY.
2010-present Co-Director, Leadership Program for Veterinary Students, College of Veterinary Medicine, Cornell University, Ithaca, NY

Other Experience and Professional Memberships

2005 Convener, American Society for Virology Meeting, Reovirus Workshop.
2007 Participant, focus group discussion on early careers of DVM clinician scientists, organized by the Burroughs Wellcome Foundation, Houston, TX.
2010-2012 Editorial Board, Journal of Virology.

Ad hoc reviewer: The Wellcome Trust, UK. (03-); Virology (03-); Journal of Virology (04-); Journal of General Virology (05-); Apoptosis (06-); Journal of Veterinary Medicine B (06-), Journal of Virological Methods (07-), Cell Biochemistry and Biophysics (07-), The Carver Trust (08-), Archives of Virology (08-), Journal of Emerging Infectious Diseases (08-); PLos Pathogens (08-); PLoS One (09-).

Professional Memberships: American Society of Microbiology; American Society for Virology

Honors

1994-1998 Graduate Research Assistantship (competitive), Cornell University.
1999-2000 NIAID, National Research Service Award, Cornell University.
2000-2002 NIAID, National Research Service Award, Harvard Medical School.
2002-2003 Mentored Clinical Scientist Development Award, Harvard Medical School.
2003-2005 Mentored Clinical Scientist Development Award, Cornell University.
2005, 2006 Outstanding Merit Award, Cornell University
2005-2010 Burroughs Welcome Fund Investigators in Pathogenesis of Infectious Disease Award.
2007 Pfizer Animal Health Award for Research Excellence.
2010-2013 Councilor for Veterinary Virology, American Society for Virology.

C. Selected Peer-reviewed publications (selected from a total of 26).

Most relevant to the current application

1. **Parker, J.S.L.**, T.J. Broering, J. Kim, D.E. Higgins, and M.L. Nibert. 2002. Reovirus core protein μ2 determines the filamentous morphology of viral inclusion bodies by interacting with and stabilizing microtubules. *J. Virol.* 76:4483-4496.
2. Broering, T.J., **J.S.L. Parker**, J. Kim, J. Jane-Valbuena, P.L. Joyce, and M.L. Nibert. 2002. Mammalian reovirus nonstructural protein μNS forms large inclusions and colocalizes with reovirus microtubule-associated protein μ2 in transfected cells. *J. Virol.* 76:8285-8297.
3. Broering, T.J., J. Kim, C.L. Miller, C.D.S. Piggot, J.B. Dinoso, M.L. Nibert, and **J.S.L. Parker**. 2004. Reovirus nonstructural Protein μNS recruits viral core surface proteins and entering core particles to Factory-like Inclusions. *J. Virol*. 78:1882-1892.
4. Coffey, C.M., A. Sheh, I.S. Kim, K. Chandran, M.L. Nibert, and J.S.L. Parker. 2006. Reovirus outer capsid protein μ1 induces apoptosis and associates with lipid droplets, endoplasmic reticulum, and mitochondria. *J. Virol.* 80:8422-8438. **Editors' selection as one of the articles of significant interest from this issue.**
5. Danthi, P., C.M. Coffey, **J.S.L. Parker**, T. W. Abel, and T.S. Dermody. 2008. Independent regulation of reovirus membrane penetration and apoptosis by the μ1 phi domain. *PLoS Pathog*. 4(12):e1000248 (14 pp). Epub 2008 Dec. 26. PMCID: PMC2600812.

Additional recent publications of importance to the field (in chronological order)

1. Miller, C.M., Broering, T.J., **J.S.L. Parker**, M.M. Arnold, and M.L. Nibert. 2003. Reovirus σNS protein localizes to inclusions through an association requiring the μNS amino-terminus. *J. Virol.* 77:4566-4576.
2. Chandran, K., **J.S.L. Parker**, M. Ehrlich, T. Kirchhausen, and M.L. Nibert. 2003. The δ region of outer-capsid protein μ1 undergoes conformational change and release from reovirus particles during cell entry. *J. Virol*. 77:13361-13375.
3. Kim, J., **J.S.L. Parker**, K.E. Murray, and M.L. Nibert. 2004. Nucleoside and RNA triphosphatase activities of orthoreovirus transcriptase co-factor μ2. *J. Biol. Chem*. 279:4394-4403.
4. Odegard, A.L., K. Chandran, X. Zhang, **J.S.L. Parker**, T.S. Baker, and M.L. Nibert. 2004. Putative autocleavage of outer capsid protein μ1, allowing release of myristoylated peptide μ1N during particle uncoating, is critical for cell entry by reovirus. *J. Virol*. 78:8732-8745.
5. Miller, C.L., **J.S.L. Parker**, J.B. Dinoso, C.D.S. Piggott, M.J. Perron, and M.L. Nibert. 2004. Increased ubiquitination and other covariant phenotypes attributed to a strain- and temperature-dependent defect of reovirus core protein μ2. *J. Virol*. 78:10291-10302.

6. Yin, P., N.D. Keirstead, T.J. Broering, M.M. Arnold, **J.S.L. Parker**, M.L. Nibert, and K.M. Coombs. 2004. Comparisons of the M1 genome segments and encoded μ2 proteins of different reovirus isolates. *BMC Virology J.* 1:6 (17 pp, http://www.virologyj.com/content/1/1/6).
7. Ossiboff, R.J., A. Sheh, J. Shotton, P.A. Pesavento, and **J.S.L. Parker**. 2007. Feline caliciviruses (FCV) isolated from cats with virulent systemic (VS) disease possess in vitro phenotypes distinct from other FCV isolates. *J. Gen. Virol.* 88:506-517.
8. Ossiboff, R.J. and **J.S.L. Parker**. 2007. Identification of regions and residues in feline junctional adhesion molecule (fJAM-A) required for feline calicivirus (FCV) binding and infection. *J. Virol.* 81:13608-13621.
9. Pesavento, P.A., K.-O. Chang, and **J.S.L. Parker**. 2008. Molecular virology of feline calicivirus. *Vet. Clin. Small Anim.* 38:775-786.
10. Ossiboff, R.J. Zhou, Y., Lightfoot, P.J., Prasad, B.V.V., and **J.S.L. Parker**. 2010. Conformational changes in the capsid of a calicivirus upon interaction with its functional receptor. *J. Virol.*84:5550-5564.

D. Research Support.

Ongoing Research Support

5 R01 AI063036-05 Parker (PI) 07/15/05 – 12/31/10 (NCE)
NIH, NIAID
Reovirus factories: structure, function, and dynamics.
The major goal of this project is to understand how cellular and viral factors interact within VFs to regulate viral assembly and replication. Specific aims: 1) to identify cellular proteins associated with VFs and their function(s) in viral assembly and/or replication; 2) to identify the role(s) of HSP70 chaperones in outer capsid μ1:σ3 heterohexamer assembly and in regulating heterohexamer recruitment to VFs; and 3) to determine the role that remodeling of the VF matrix plays in assembly of the double-layered particle.
Role: PI

[redacted]

Completed Research Support (last 3 years)

[redacted]

[redacted]

[redacted]

[redacted]

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Colgate University

Delete Entry *** Start Date:** 04/01/2011 *** End Date:** 03/31/2014 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Geoffrey	Howard	Holm		PD/PI	[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
2.		TBD		TBD		Technician		[redacted]			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person [redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
[redacted]	Undergraduate Students			[redacted]	[redacted]	[redacted]	[redacted]
	Secretarial/Clerical						
[redacted]	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Colgate University

Delete Entry *** Start Date:** 04/01/2011 *** End Date:** 03/31/2014 **Budget Period 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.	Dual water-jacketed CO2 incubators	[redacted]
2.	Sonicator with sound enclosure	[redacted]
3.	-80C freezer	[redacted]
4.	Refrigerated microcentrifuge + rotor	[redacted]
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	[redacted]

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	[redacted]
4.	Subsistence	
5.	Other	
[redacted]	**Number of Participants/Trainees** **Total Participant/Trainee Support Costs**	[redacted]

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Colgate University

Delete Entry * Start Date: 04/01/2011 * End Date: 03/31/2014 Budget Period 1

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Salary and Wages	[redacted]	[redacted]	[redacted]
2.			
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)
	0.00

K. * Budget Justification 1240-R&R BUDGET JUSTIFICATION.pdf

Add Attachment Delete Attachment View Attachment

(Only attach one file.)

BUDGET JUSTIFICATION

Personnel Justification

Holm, Geoffrey H., Ph.D., Principle Investigator

- 80% summer effort (mid-May through mid-August) (~2.0 summer months in Years 1,2 & 3)
- 100% effort during junior faculty leave (~4.0 academic months in Year 2)
- Salary based on current base of $[redacted] with 2.2% increase per year.
- Fringe benefit rate is 27.9%

The PI will have primary responsibility for overseeing student research and training activities, analysis and interpretation of results, and preparation of manuscripts reporting on the work. He will generate most of the reovirus reassortants and mutants necessary for the proposed experiments, and perform experiments where appropriate. He will also train and supervise the work of the research technician.

Research technician (half-time, Years 1,2 & 3)

- 100% calendar year effort for half-time position (6.0 person months)
- 20 hours a week @ $[redacted]/hour for 48 weeks
- Fringe benefit rate is 10%

The research technician will be responsible for management of the lab, including maintaining cell lines and virus stocks and facilitating student research. The technician will also perform or assist with experiments as appropriate.

Undergraduate student research assistant #1

- 100% summer effort (2.5 person months per year in Years 1,2 & 3)
- 10 weeks @ $[redacted] per week in year 1; 10 weeks @ $[redacted] per week in years 2 & 3.
- Fringe benefit rate is 10%

Undergraduate research assistant will be recruited from Microbiology, Virology or Immunology courses, and will perform experiments to determine the phenotypes of generated reovirus reassortants and mutants on innate immune signaling.

Undergraduate student research assistant #2

- 100% summer effort (2.5 person months per year in Years 1,2 & 3)
- 10 weeks @ $[redacted] per week in year 1; 10 weeks @ $[redacted] per week in years 2 & 3.
- Fringe benefit rate is 10%

Undergraduate research assistant will be recruited from Microbiology, Virology or Immunology courses, and will perform experiments to define the cellular transcriptional networks induced in response to reovirus reassortants.

Parker, John, B.V.M.S., Ph.D., Other Significant Contributor

- Effort of 0 person months; as needed.

Dr. Parker will facilitate access to equipment at Cornell University that is unavailable at Colgate University, and provide technical support and assistance, when required.

Major Equipment Justification (Colgate University rules as >$[redacted]

Dual water-jacketed CO2 incubators: ($[redacted] + shipping) Required for tissue culture. Replaces 1 ~20 year old Fisher Isotemp incubator. Dual incubators allows for separation of clean and virus-infected tissue culture.

Cell sonicator and sound enclosure: ($[redacted] + shipping) Required for reovirus purification. Replaces ~20 year old sonicator.

-80°C freezer: ($[redacted] + shipping) Required for storage of virus and bacterial stocks and molecular biology reagents. Alleviates current lack of storage space issues in shared freezers.

Refrigerated microcentrifuge + rotor: ($[redacted] + shipping) Required for molecular biology protocols. Replaces 1 ~20 year old non-refrigerated microcentrifuge.

Travel Justification: ($[redacted] PI + $[redacted] student) Funds requested for PI and undergraduate student travel to annual meetings of the American Society of Virology or the American Society of Microbiology (1 meeting/yr).

Materials and Supplies Justification: ($[redacted]

Consumables: (~$[redacted]/year) Includes tissue culture media and reagents, fetal bovine serum, tissue culture plates, pipettes, etc.

Molecular biology reagents: (~$[redacted]/year) Includes cell death detection kits, luciferase assay kits, cDNA synthesis and labeling kits, PCR and qPCR enzymes, DNA sequencing enzymes, DNA and RNA purification kits, primary and secondary antibodies, etc.

Chemicals: (~$[redacted]/year) Includes salts and other buffers, components for bacterial culture broths, antibiotics, etc.

Supplies for Biol 373 laboratory component: (~$[redacted] in years 1 and 3) Includes tissue culture media and molecular biology reagents specifically dedicated to running the proposed laboratory component of Biol 373: Virology.

Publication Costs Justification: ($[redacted] Estimated costs for ~3 publications in a journal such as the *Journal of Virology*.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	[redacted]	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		[redacted]
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign		
Section E, Participant/Trainee Support Costs		[redacted]
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel	[redacted]	
4. Subsistence		
5. Other		
6. Number of Participants/Trainees	[redacted]	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	[redacted]	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		[redacted]

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Geoffrey
Middle Name: Howard
* Last Name: Holm
Suffix:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Mr.
* First Name: Bruce
Middle Name:
* Last Name: Moseley
Suffix:

* Phone Number: [redacted]
Fax Number:
Email: [redacted]

* Title: Associate Director, CFGR

* Street1: 13 Oak Drive
Street2:
* City: Hamilton
County/Parish: Madison
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 13346-1338

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1241-R15 AREA grant specifi	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1242-R15 AREA research plan	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1249-VERTEBRATE ANIMALS.pdf	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1250-Letters of support.pdf	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments Remove Attachments View Attachments

SPECIFIC AIMS

Mammalian orthoreoviruses serve as tractable experimental models for studies of viral pathogenesis. Diseases caused by reovirus in mammalian hosts are strain-specific: type 1 viruses are associated with myocarditis, whereas type 3 viruses cause lethal encephalitis. The molecular mechanisms by which reoviruses cause these disparate diseases are not fully understood. However, pathologic hallmarks of these diseases are recapitulated in cell-culture models of reovirus infection. Encephalitis induced by type 3 reovirus correlates with its capacity to induce apoptosis both *in vivo* and in cell culture, whereas myocarditis caused by type 1 reovirus is associated with its limited capacity to induce type 1 interferons (IFNs), a family of related antiviral cytokines. Both of these phenotypes are linked to innate immune responses in reovirus-infected cells. However, our preliminary data suggest that IFN-induced signaling is not required for reovirus-induced apoptosis, suggesting that these phenotypes are distinct consequences of innate immune recognition of reovirus infection. **The primary goal of the proposed research is to determine mechanisms underlying reovirus strain-specific differences in the activation of innate immune responses leading to apoptosis and IFN induction.** To attain this goal, I propose the following specific aims:

Specific Aim 1. To define the mechanism for reovirus strain-specific differences in the activation of innate immune signaling pathways.

Prototype reovirus strains type 1 Lang (T1L) and type 3 Dearing (T3D) differ in the capacity to activate innate immune transcription factor IRF-3 (Research Strategy, Figs. 2 and 3). We envision two possible explanations for the observed differences. First, differences in capsid stability following cell entry may lead to variable cytoplasmic exposure of genomic double-stranded RNA (dsRNA), a known agonist of innate immune signaling. Second, reovirus strains may encode an antagonist that interferes with signaling pathways upstream of IRF-3 activation. Experiments proposed in this specific aim will discriminate between these two possibilities by determining the effect of known capsid-destabilizing mutations on IRF-3 activation. Single-gene reovirus reassortants generated using a recently developed reverse genetics system will be used to identify genes associated with differences in innate immune antagonism. These experiments will define molecular mechanisms of innate immune response activation by a dsRNA virus.

Specific Aim 2. To identify reovirus strain-specific IFN-dependent and -independent transcriptional networks.

Reovirus IFN expression and apoptosis induction are linked to activation of innate immune transcription factors IRF-3 and NF-κB. Preliminary data suggest that these pathways are discrete, as IFN signaling is not required for reovirus-induced apoptosis (Research Strategy, Figs. 4 and 5). IFN induction and apoptosis will be segregated using reovirus reassortants that lack the capacity to engage these pathways, and the transcriptional responses required for each pathway will be defined by microarray analysis. These experiments will be facilitated by the GCAT Consortium, which aims to bring genomics methods into the undergraduate curriculum through student research.

Specific Aim 3. To integrate investigative research addressing reovirus innate immune responses into an undergraduate biology curriculum.

The biology curriculum at Colgate University values investigative laboratory experiences. We provide students with training in the methodologies and practice of scientific investigation through laboratory courses and independent study. These opportunities will be used to facilitate investigation into reovirus innate immune responses. To accomplish this goal, I will develop an investigative laboratory component for an upper-level virology elective. This laboratory experience will engage students in a semester-long project, first generating reovirus reassortants or mutants using reverse genetics, followed by analysis of innate immune signaling. Additionally, I will continue to incorporate upper-level undergraduate students into my research program through my research course, independent study experiences, and summer research.

Completion of these specific aims will result in the publication of at least two manuscripts, featuring multiple undergraduate coauthors. Broadly, the experimental results will enhance understanding of how genetically similar viruses can cause disparate diseases through manipulation of innate immune pathways. This research also will provide insight into two long-standing questions in the dsRNA virus field: mechanisms of apoptosis and mechanisms activation and evasion of cellular antiviral responses. As other pathogenic microbes employ similar strategies, the proposed experiments will uncover conserved mechanisms of infectious disease pathogenesis. Finally, these aims will enhance Colgate's mission to provide undergraduate students with opportunities for significant independent research in preparation for careers in science and medicine.

RESEARCH STRATEGY

1. SIGNIFICANCE

1.1. INTRODUCTION

The interface between viral infection, the innate immune response, and cellular apoptotic machinery is a critical nexus of disease pathogenesis. The innate immune system responds to viral infection by creating an antiviral state in infected cells and surrounding tissue. In response to these pressures, viruses have evolved multiple strategies for subverting innate immunity, which center on antagonizing innate immune pathways or manipulating cell death signaling downstream of innate immune recognition. Although general features for each of these processes in isolation are for the most part known, molecular mechanisms by which these processes are regulated in concert at the cellular level have not been fully elucidated. The proposed research uses mammalian reovirus, a highly tractable experimental model, to identify novel mechanisms of innate immune response antagonism and to determine their effects on cellular transcriptional networks that influence the antiviral response and apoptosis.

Reovirus exhibits strain-specific differences in diseases caused in mammalian hosts. Some type 1 viruses are associated with myocarditis, whereas type 3 viruses cause lethal encephalitis. Both viruses utilize the same receptor for cell entry in most cell types; therefore, differences in disease pathogenesis are dependent upon other mechanisms, including the intrinsic cellular responses elicited by these viruses. We have generated preliminary data that indicates that: i) prototype strains type 1 Lang (T1L) and type 3 Dearing (T3D) exhibit differences in their capacity to activate transcription factor interferon regulatory factor-3 (IRF-3) and ii) IFN signaling and apoptosis induction following reovirus infection are separate and discrete consequences of activation of IRF-3 and nuclear factor-κB (NF-κB). These studies provide a strong experimental foundation to ask the following questions: What are the molecular mechanisms responsible for differences between T1L and T3D in IRF-3 activation? What viral genetic determinants dictate these differences? What cellular transcriptional networks are responsible for inducing an antiviral response versus apoptosis following reovirus infection? The central hypothesis of the proposed research is that T1L encodes an antagonist of IRF-3 activation that alters regulation of cellular transcriptional networks that establish an antiviral state or induce apoptosis. The proposed experiments will test this hypothesis and enhance understanding of how genetically similar viruses can cause disparate diseases through manipulation of innate immune pathways. Importantly, the experiments described in this proposal will be performed by undergraduate student researchers and will be facilitated by development of a laboratory component for a Virology course that will provide biology students with exposure to original research questions, virologic techniques, and the scientific process.

1.2. REOVIRUS REPLICATION AND PATHOGENESIS

Reoviruses are nonenveloped, icosahedral viruses that contain a genome of 10 dsRNA gene segments (1). Virions are formed from two concentric protein shells, called outer capsid and core. The outer capsid is composed primarily of the σ3 and μ1 proteins, whereas the inner core is composed primarily of λ1 and σ2 (Fig. 1). Reovirus entry into cells is initiated by the attachment of the σ1 protein to cell-surface receptors (2, 3). The σ1 protein of both type 1 and type 3 reovirus binds the receptor junctional adhesion molecule-A (JAM-A) (4-6). After receptor binding, virions are internalized into endosomes (7-10), which serve as the site of disassembly. During the disassembly process, σ1 and σ3 are lost, and μ1 is cleaved to form the infectious subviral particle (ISVP) (11-13). The μ1 cleavage fragments undergo conformational rearrangement to penetrate endosomes and deliver transcriptionally active core particles into the cytoplasm (14, 15). Cytoplasmic entry is required for subsequent stages of the virus replication program, including induction of the antiviral response and apoptosis (16-19).

Virtually all mammals, including humans, serve as hosts for reovirus infection. Reoviruses are highly virulent in newborn mice, causing disease in several organs, including the central nervous system (CNS) and heart (1). Reovirus encephalitis is associated with apoptosis of infected neurons, and is dependent on the capacity of reovirus strains to induce apoptosis (20-23). In the heart, reovirus pathogenesis is tightly associated with the antiviral response, as myocarditis is associated with strains that induce lower levels of IFN-β and are less sensitive to its effects (22, 24). Reovirus exhibits strain-specific differences in the capacity to induce both apoptosis and IFN. T3D is a potent inducer of apoptosis, whereas T1L has a significantly reduced capacity to activate cell death pathways (25-27). This phenotype

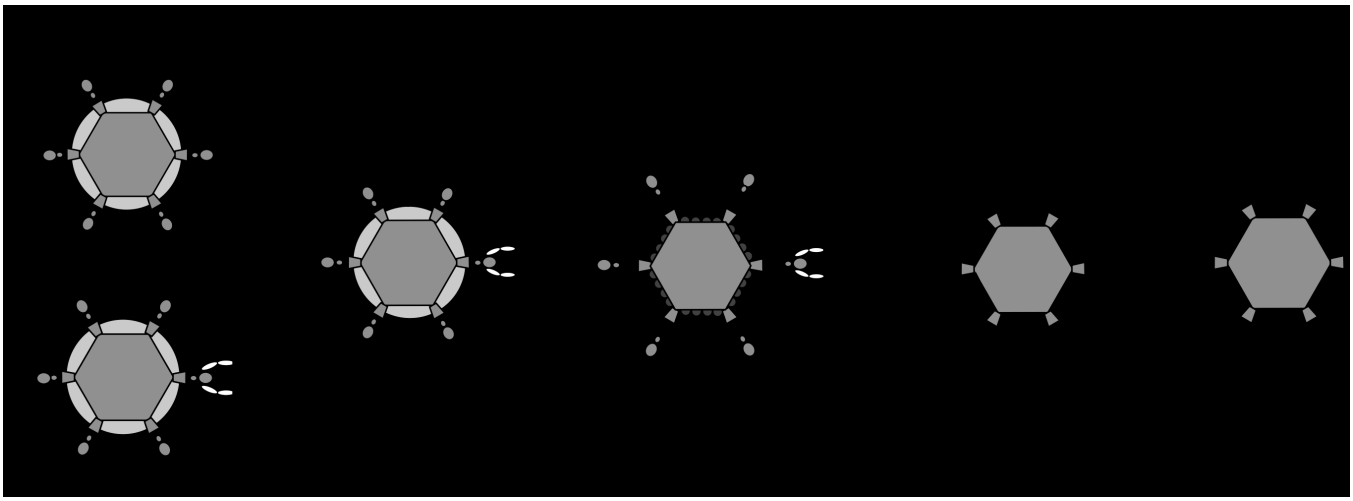

Fig. 1. Stepwise disassembly of reovirus. (a) Following attachment to cell-surface carbohydrate (sialic acid [SA] for type 3 reoviruses) and JAM-A, reovirus virions enter cells by receptor-mediated endocytosis (b). In the endocytic compartment, the viral outer capsid undergoes acid-dependent proteolysis. (c) The first disassembly intermediate is the ISVP, which is characterized by loss of σ3 and cleavage of μ1 into particle-associated fragments δ and ϕ. (d) The ISVP then undergoes further conformational changes to form the ISVP*. The ISVP* is characterized by conformational rearrangements of the μ1 protein fragments to expose hydrophobic residues and loss of attachment protein σ1. (e) The μ1 cleavage fragments mediate viral penetration of the endosomal membrane, releasing the transcriptionally active core, composed primarily of λ2, λ3, and σ2, into the cytoplasm.

was originally ascribed to the σ1 attachment protein and the outer capsid protein μ1 (25). Recent evidence suggests that the primary determinant of apoptosis is μ1, downstream of sialic acid/JAM-A/σ1-dependent entry of virions into endosomes (18, 23, 26-28). T3D also induces significantly higher levels of IFN-β than T1L, which segregates to the M1, S2, and L2 genes (24). The molecular mechanisms that determine these differences are unknown.

1.3. INNATE IMMUNE RECOGNITION OF REOVIRUS INFECTION

Cells possess a wide array of sensors to detect the presence of invading pathogens. The RNA helicases RIG-I and Mda-5 recognize cytoplasmic dsRNA and initiate signaling cascades via a downstream adaptor, IPS-1 (VISA/MAVS/Cardif), that activate IRF-3 and NF-κB (29). These transcription factors induce the production of type I IFNs and other proinflammatory cytokines. In turn, type I IFNs signal through the IFN-α/β receptor (IFNAR) to induce expression of multiple interferon stimulated genes (ISGs), such as protein kinase R (PKR) and 2'5' oligoadenylate synthetase (OAS), which mediate antiviral, immunomodulatory, and cell cycle inhibitory effects (30).

RIG-I and Mda-5 have both been implicated in recognition of reovirus infection (16, 31, 32). Recent experiments defining the specific molecular inducers of these proteins indicate that RIG-I activation requires short segments of dsRNA containing a 5' triphosphate moiety, whereas Mda-5 activation requires long segments of dsRNA (33-35). Given the requirement for cytoplasmic dsRNA for activation of these proteins, it remains unclear how they access reovirus dsRNA. Upon penetration of the reovirus core into the cytoplasm, genomic dsRNA segments remain inside the core and are copied during primary transcription to produce capped (+)-strand mRNAs, which are extruded from the core (36-38). Negative strand synthesis does not occur until newly synthesized virions are completed and have packaged a (+)-strand RNA (39-41). These data suggest that genomic dsRNA is never exposed in the cytoplasm. However, recent immunofluorescence experiments using an anti-dsRNA antibody indicate that immunodetectable dsRNA is present in reovirus-infected cells (35) and is capable of eliciting an innate immune response.

Reovirus activates IRF-3 and NF-κB in HeLa cells by 2-4 h post-infection, and stimulates maximal transcription factor activity by 8-12 h (16, 42, 43). NF-κB subunits p50 and p65 are activated via a mechanism involving IκBα, IKKα, and IKKγ (42, 43). IRF-3 activation is dependent upon RIG-I, Mda-5, and IPS-1 (16, 31, 32). Importantly, siRNA knockdown of RIG-I or IPS-1 does not inhibit NF-κB activation (16), which suggests that reovirus engages the NF-κB pathway via an alternative mechanism. Both NF-κB and IRF-3 are required for efficient induction of apoptosis in reovirus-infected cells (16, 22, 42, 43), and for induction of IFN-β in cultured cells and *in vivo* (16, 22, 44, 45). Microarray profiling of NF-κB-dependent

genes induced by reovirus infection showed temporal expression of ISGs, but failed to identify genes that strongly link NF-κB activation with host cell death pathways (45). This may be due to overwhelming induction of IFN pathways, which may mask initial induction of apoptosis-inducing genes, or attributable to the particular time points profiled. Therefore, transcriptional networks responsible for apoptosis initiation remain unknown.

1.4. PRELIMINARY STUDIES (**, data generated by undergraduate students)

1.4.1. REOVIRUS EXHIBITS STRAIN-SPECIFIC DIFFERENCES IN THE CAPACITY TO ACTIVATE INNATE IMMUNE RESPONSES

Molecular mechanisms responsible for differences in the capacity of T1L and T3D to induce IFN-β are not completely understood. Recently, we showed that the μ2 protein of T1L inhibits signaling downstream of the type I IFN receptor (IFNAR) (46). This may account for differences in IFN-β induction, since IFNAR signaling initiates a positive feedback loop, through the production of IRF-7, which increases transcription of IFN-β (47, 48). To test whether strain-specific differences in IFN induction also occur *prior* to IFN-dependent signaling, we assessed the induction of IFN-β mRNA by quantitative RT-PCR (qPCR). Infection with T3D rapidly and strongly induced IFN-β mRNA, by 6 h post-infection, whereas infection with T1L induced much lower levels of IFN-β, by 12 h post-infection (Fig. 2). The timing of this disparity in induction is too rapid to be explained solely by inhibition of the IFNAR feedback loop, indicating that T1L and T3D exhibit differences in their capacity to induce primary IFN-β expression.

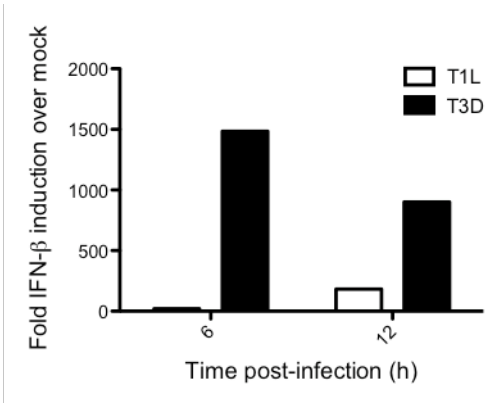

****Figure 2. T1L and T3D exhibit differences in their capacity to induce IFN-β at early times post-infection.** HeLa cells were infected with T1L and T3D at an MOI of 100 pfu/cell. RNA was harvested from cells at the indicated times post-infection, and used to generate cDNA. IFN-β mRNA expression was determined by qPCR, normalized to GAPDH expression and to mock-infected cells.

We next examined activation of the IFN-β-inducing transcription factors IRF-3 and NF-κB following reovirus infection. HeLa cells were infected with T1L and T3D, and cells were fixed 6 h post-infection and immunostained using antibodies against IRF-3 and NF-κB. The percentage of cells exhibiting nuclear localization of these transcription factors was quantified by determining the pixel density overlap of the IRF-3- or NF-κB-dependent signal with DAPI-stained nuclei using confocal microscopy. Whereas no difference was observed in the levels of nuclear NF-κB between T1L- and T3D-infected cultures, T3D-infected cultures exhibited significantly higher levels of nuclear IRF-3 (Fig. 3). These data are consistent with previous data that type 1 and type 3 reoviruses activate NF-κB at similar levels at early times post-infection (49), and support our observation that reoviruses induce NF-κB by a distinct mechanism from IRF-3 (16). This also suggests that differences in the level of IFN-β induced by T3D and T1L are the result of a differential capacity of these viruses to activate IRF-3, which occurs via a RIG-I/Mda-5/IPS-1-dependent pathway (16, 31) and is independent of IFNAR signaling. Experiments proposed in **Specific Aim 1** will determine the mechanism for differences in IRF-3 activation following T1L and T3D infection.

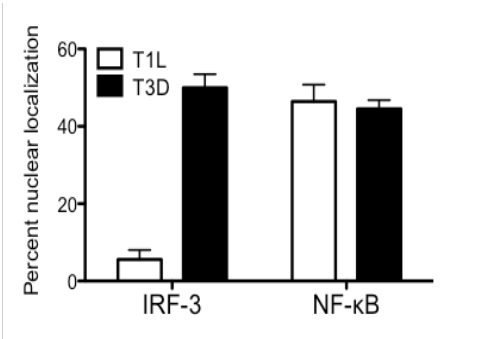

****Figure 3. T1L and T3D exhibit differences in IRF-3 activation at early times post-infection.** HeLa cells were infected with T1L and T3D at an MOI of 100 PFU/cell. At 6 h post-infection, cells were fixed and immunostained using anti-IRF-3 and anti-NF-κB p65 antibodies. The percentage of cells exhibiting nuclear localization of IRF-3 or NF-κB was quantified using confocal microscopy.

1.4.2. IFN SIGNALING IS NOT REQUIRED FOR REOVIRUS APOPTOSIS

Analysis of NF-κB-dependent genes induced by reovirus identified many genes involved in the innate immune response, including many interferon-stimulated genes (ISGs) (45). These results raised the possibility that the IFN signaling pathway itself was sufficient to trigger apoptosis in reovirus-infected cells. We

have generated three lines of evidence that IFN signaling is not required for apoptosis of reovirus-infected cells. First, exogenous IFN-β did not restore the capacity of reovirus to induce apoptosis in IRF-3-deficient murine embryonic fibroblasts (MEFs), which do not produce their own IFN (Fig. 4). Second, an anti-IFN-β antibody, which blocks IFN signaling, did not inhibit apoptosis in wild type cells (Fig. 4). Third, reovirus retained the capacity to induce apoptosis in IFNAR-deficient MEFs (Fig. 5). These data suggest that although NF-κB and IRF-3 are required for both IFN induction and apoptosis initiation, these phenotypes are evoked by separate and distinct pathways. Experiments proposed in **Specific Aim 2** will identify the IFN-dependent and -independent transcriptional networks induced by T1L and T3D that induce these disparate phenotypes.

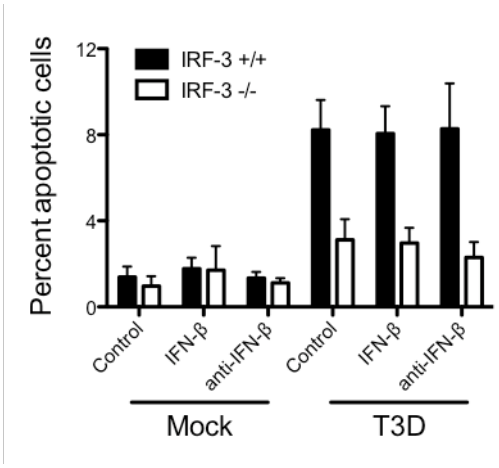

Figure 4. IFN signaling is not required for reovirus apoptosis. IRF-3 +/+ or -/- MEFs were mock-infected or infected with reovirus T3D at an MOI of 100 PFU/cell in the presence or absence of exogenous IFN-β (1000 I.U.) or anti-IFN-β antibody. Cell death was quantified by acridine orange staining 24 h post-infection.

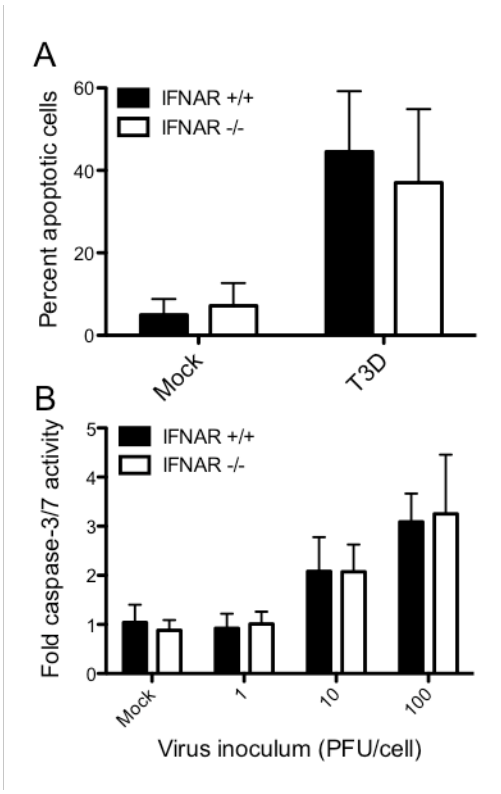

Figure 5. IFN signaling is not required for reovirus apoptosis. IFNAR +/+ or -/- MEFs were mock infected or infected with reovirus at an MOI of 100 PFU/cell (A) or as indicated (B). Cell death was quantified by acridine orange staining (A) or by caspase activity (B) 24 h post-infection.

1.4.3. MICROARRAY PROFILING OF REOVIRUS μ2-DEPENDENT ALTERATIONS IN CELLULAR TRANSCRIPTIONAL NETWORKS

The identification of the T1L μ2 protein, encoded by the M1 gene, as an antagonist of IFNAR signaling (46) provides an opportunity to decouple IFN signaling from apoptosis induction and identify transcriptional networks that are required for each phenotype. We generated T1LxT3D M1 reassortants and confirmed the M1 phenotype by sequencing and by immunofluorescence microscopy to examine replication complex morphology (not shown). We performed a pilot experiment to examine transcriptional networks activated by these reassortants and the parental controls using microarray slides obtained through the Genome Consortium for Active Teaching (GCAT; www.bio.davidson.edu/GCAT). GCAT is a consortium of faculty at primarily undergraduate institutions that aims to facilitate undergraduate research using genomics methods (see letter from Dr. M. Campbell). HeLa cells were mock infected or infected in triplicate with rsT1L, rsT3D, rsT1L-T3DM1, and rsT3D-T1LM1, and RNA was extracted from cells at 12 h post-infection. This time point was chosen for initial studies because it is subsequent to the initial induction of IFN, but prior to robust activation of effector caspases that carry out the apoptotic program. RNA was converted to cDNA using the Array 350 kit (Genisphere) and directly compared with pooled cDNA collected at the time of initial infection (0 h), with reciprocal dye swaps between the triplicate samples. Mixed cDNA was hybridized to human HEEBO microarray slides and labeled with the 3DNA capture reagent (Genisphere). Labeled microarray slides were then scanned at one of the GCAT scanning facilities. Although analysis of this data set is not complete, we have preliminarily identified subsets of genes differentially regulated following infection with these viruses, and used these to perform gene ontology analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) software available through the NIAID (50) (Table 1). These data indicate that this approach to identify IFN-dependent and -independent genes induced by reovirus is feasible and accessible to undergraduate students. Experiments proposed in **Specific Aim 2** will extend the microarray analysis to earlier and later time points post-infection, and validate identified target genes for their role in reovirus apoptosis.

**Table 1: Gene ontology analysis of genes induced by M1 reassortants

T3D	T1L
Response to virus	Gene regulation by peroxisome proliferators via PPARα
Cell motion	Positive regulation of cell differentiation
Cytosolic DNA-sensing pathway	DNA replication, recombination, and repair
Cytoskeletal protein binding	Regulation of transcriptional activity by PML
Actin-binding	Chaperones modulation of interferon signaling pathway
Transcription regulation	Response to retinoic acid
Toll-like receptor signaling pathway	Transcription coactivator activity
Defense response	Regulation of anti-apoptosis
Antiviral defense	Cell motion
Inflammatory response	
rsT3D-T1LM1	**rsT1L-T3DM1**
Response to virus	DNA replication, recombination, and repair
Cell adhesion molecules (CAMs)	Nucleotide phosphate-binding region:ATP
Regulation of nitrogen compound metabolism	RNA binding
Transcription factor activity	Cytosolic DNA-sensing pathway
Immune response	I-kappaB kinase/NF-kappaB cascade
Transcription regulator activity	Antiviral defense
Zinc finger, nuclear hormone receptor-type	Ubiquitin thiolesterase activity

1.5. SIGNIFICANCE OF THE PROPOSED RESEARCH

The primary objective of the proposed research is to elucidate mechanisms by which reovirus interfaces with innate immune pathways and determine their functional consequences on cellular transcriptional networks (Fig. 6). These cellular response networks are key determinants of strain-specific differences in reovirus pathogenesis in the CNS and heart. Identifying these networks will further define the process by which an infected cell either enters an antiviral state or undergoes apoptosis, and facilitate understanding of how genetically similar viruses can cause disparate diseases by manipulating cellular signaling pathways. As other pathogenic microbes elicit similar responses, the proposed experiments will uncover broadly conserved mechanisms of infectious disease pathogenesis, with particular relevance for viral encephalitis and myocarditis. This research will also provide insight into how reoviruses activate and evade innate immune pathways, which are long-standing questions in the dsRNA virus field. Finally, this application will significantly enhance the undergraduate research program at Colgate University. It will provide opportunities for students to gain practical experience with modern virology, through a new laboratory component for an upper-level Virology course and through independent research projects in my lab, in preparation for careers in biomedical research.

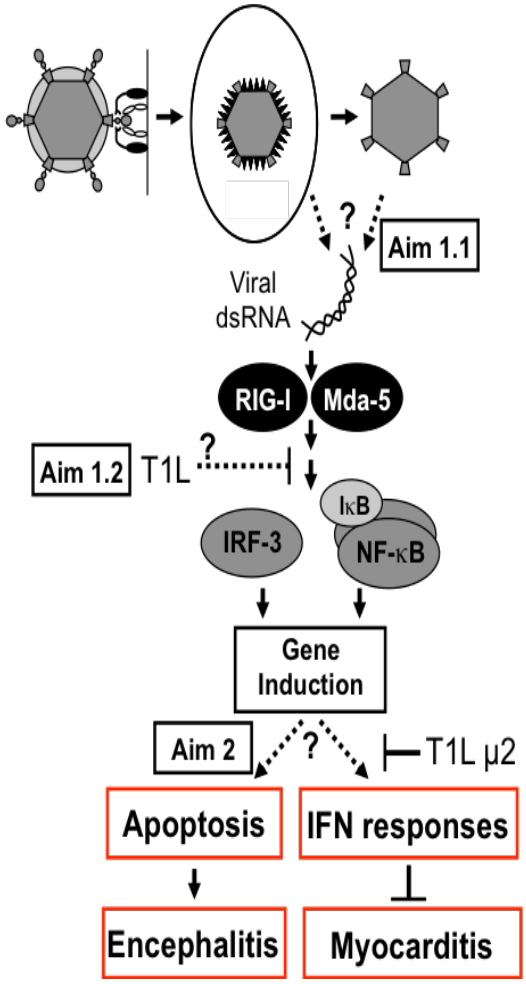

Figure 6. Model for mechanisms and functional consequences of reovirus innate immune activation. During or shortly after virus penetration into the cytoplasm, genomic dsRNA becomes exposed to the sensors RIG-I and Mda-5. Experiments proposed in **Specific Aim 1.1** will investigate molecular mechanisms of cytoplasmic dsRNA exposure. Reovirus T3D activates IRF-3 to a significantly higher level than T1L, indicating that T1L may encode an antagonist of signaling pathways leading to IRF-3 activation. This possibility will be explored in **Specific Aim 1.2.** Both IRF-3 and NF-κB are required for IFN induction and apoptosis following reovirus infection; however, the transcriptional networks responsible for these phenotypes are distinct. Experiments proposed in **Specific Aim 2** will define these networks by microarray analysis, using the T1L μ2 protein, which antagonizes IFN signaling, to decouple apoptotic signaling from IFN responses. These experiments will elucidate mechanisms of reovirus pathogenesis in the CNS and heart.

2. INNOVATION

Much of the innovation in this proposal lies in the synergy between specific aims directed at providing an outstanding and productive research opportunity in viral innate immune responses for undergraduate students. Few programs exist at liberal arts colleges that expose students to rigorous virology research. The proposed grant will

provide Colgate students with a unique opportunity to engage in meaningful scientific research, while gaining experience in modern virologic and molecular genomics techniques. The reovirus model is perfect for introducing undergraduates to virology. Reovirus is safe and easy to work with, and reagents are readily available. There are well-established written protocols and an extensive, but not overwhelming, literature. Additionally, the long history of research on reovirus pathogenesis using the mouse model fuels the interest of many students who are anticipating future careers in biomedical research. Students will be first introduced to this model system while taking the proposed laboratory component for Biol373: Virology. These laboratory modules will generate virus reagents, probe mechanisms of reovirus-induced innate immune signaling, and expose students to many of the techniques used in my research laboratory. These students may then join my research program, either as summer research assistants or through my upper-level research course, and be well prepared to undertake an independent research project. Many of the techniques proposed in the application are robust and well tested, out of necessity for use in an undergraduate research setting. Nevertheless, the research questions asked are significant, and make use of the resources, such as GCAT, that are available at primarily undergraduate institutions to facilitate research of this kind. The pairing of these microarray profiling resources with the recently developed reovirus reverse genetics system (51, 52) provides a powerful tool for dissecting molecular mechanisms by which reovirus engages cellular antiviral responses and apoptotic pathways, in a context that is accessible to undergraduate students.

3. APPROACH

3.1. SPECIFIC AIM 1. *To define the mechanism for reovirus strain-specific differences in the activation of innate immune signaling pathways.*

3.1.1. GENERAL EXPERIMENTAL APPROACH.

Type 3 reovirus infection induces innate immune responses leading to activation of IRF-3 by 4-6 h post-infection (16, 42, 43). In contrast, T1L infection elicits markedly diminished IRF-3 activation at early times post-infection (Preliminary Data, Section 1.4.1) and much lower levels of IFN-β expression in infected cultures (24). **The objective of experiments proposed in this specific aim is to determine the molecular mechanisms responsible for differences in innate immune response activation by type 1 and type 3 reovirus.** These experiments will be conducted in two parts. First, we will examine mechanisms of cytoplasmic dsRNA exposure leading to RIG-I- and Mda-5-dependent activation of IRF-3, using biochemical treatments of virions and known particle-destabilizing mutations. We will then examine two possible mechanisms for differences in IRF-3 activation by T1L and T3D: i) lower levels of cytoplasmic genomic dsRNA exposure following T1L infection, preventing RIG-I or Mda-5 activation; or ii) a T1L-encoded antagonist of signal transduction pathways upstream of IRF-3 activation. To differentiate between these possibilities, we will initially use single gene T1LxT3D reassortant viruses to identify genes associated with differences in innate immune activation. The results from these experiments will inform research to characterize differences in capsid stability or to identify T1L innate immune antagonists. These experiments will provide insight into how dsRNA viruses activate innate immune responses, and may identify novel reovirus IFN antagonists.

3.1.2. THE ROLE OF CAPSID STABILITY IN ACTIVATION OF INNATE IMMUNE RESPONSES FOLLOWING REOVIRUS INFECTION.

3.1.2.1. Rationale. Reovirus particles that lack genomic dsRNA fail to elicit innate immune responses (16, 53), indicating these pathways are not induced by signals caused by the binding of reovirus particles to cell surface receptors. In contrast, reovirus genomic dsRNA is sufficient to activate IRF-3 (16, 32). Since IRF-3 is activated by 2-4 h post-infection (16), dsRNA exposure likely occurs directly upon core entry into the cytoplasm and not during the particle assembly phase, which occurs much later. Additionally, IRF-3 activation is not inhibited by ribavirin, suggesting that primary transcription and subsequent steps in reovirus replication are not required (16). Finally, UV-irradiation of reovirus virions does not inhibit IFN induction (53) and can even enhance the potency of reovirus as an IFN inducer, indicating that UV treatment may destabilize the viral particle leading to increased cytoplasmic exposure of genomic dsRNA (54). Therefore, alterations in particle stability, occurring either during endosomal disassembly or after penetration of the core into the cytoplasm, might lead to genomic dsRNA exposure and subsequent activation of innate immune responses. Experiments proposed here will test this hypothesis and determine steps in the reovirus entry pathway at which genomic dsRNA exposure occurs.

3.1.2.2. Steps in reovirus entry leading to genomic dsRNA exposure. Aberrant disassembly of virions in endosomes during entry may lead to proteolytic disruption of the core and subsequent exposure of genomic dsRNA in the cytoplasm. Disassembly is regulated by the σ3 outer capsid protein, and by the membrane penetration protein μ1. Alternatively, differences in core stability following endosomal membrane penetration may also influence exposure of dsRNA in the cytoplasm. To differentiate between these possibilities, we will take advantage of the capacity of reovirus ISVPs, which lack σ3 and have an altered μ1 conformation, to bypass steps in endosomal disassembly (11-13, 55). ISVPs are capable of inducing IRF-3 activation (16), but the relationship between ISVP formation and IRF-3 activation has not been carefully examined. To characterize this relationship, the length of chymotrypsin treatment used to generate ISVPs will be varied, and these ISVPs will be examined for their capacity to activate IRF-3 by immunofluorescence microscopy, as described (Section 1.4.1), or by native PAGE followed by immunoblotting for dimeric IRF-3, as performed previously (16). As the σ1 protein of T3D is susceptible to protease cleavage, we will utilize a non-cleavable variant, recombinant strain (rs)T3D-S1T249I, to generate ISVPs (51) For these experiments, virion and ISVP protein content will be monitored by SDS-PAGE followed by Coomassie blue staining.

To assess the contribution of events subsequent to σ3 cleavage on innate immune activation, we will utilize *in vitro*-generated cores (55, 56). Cores are non-infectious and are incapable of inducing IFN response when added directly to cells (53). However, transfection techniques are capable of delivering cores to the cytoplasm and restoring infectivity (57). To assess the role of core stability, cores will be transfected into cells over a range of MOI and their capacity to induce IRF-3 will be assessed. Transfection efficiency will be monitored by fluorescence focus assay to detect virus replication complexes. Cores derived from reovirus top component, which lack dsRNA, will be used to control for transfection-dependent alterations in cellular innate immune activation. If aberrant endosomal disassembly events are responsible for IRF-3 activation, we expect that transfection of cores will fail to activate IRF-3.

3.1.2.3. The effect of defective interfering particles on IRF-3 activation. Purified reovirus virions exhibit a particle:plaque-forming unit (PFU) ratio of between 50:1 and 500:1 (58). Defective interfering particles are known to induce interferon in other virus systems (59, 60). To assess the role of these particles, we will treat purified virions with UV irradiation, which causes a dose-dependent decrease in particle:PFU ratio (61). We will then analyze the capacity of these stocks to activate IRF-3, comparing infections normalized for particle number with infections normalized for PFU. If the amount of non-infectious particles influences IRF-3 activation, we anticipate that normalizing stocks for particle:PFU ratio should alleviate these differences. In contrast, if intrinsic virion stability causes differential IRF-3 activation, then virions should activate IRF-3 independent of particle:PFU ratio.

3.1.2.4. Virion-destabilizing mutations. In a complementary approach, reovirus mutants with known alterations in capsid stability will be used to determine virion components associated with innate immune activation. Stability of the outer capsid is largely determined by sequences in σ3 (62-64). One σ3 mutation (Y354H) is particularly associated with enhanced sensitivity to protease degradation (65, 66). The effect of this mutation on innate immune activation is not known. rsT3D-S4Y354H will be generated and tested for its capacity to activate IRF-3 in comparison to parental virus rsT3D. If aberrant virion degradation by endosomal proteases is linked to dsRNA exposure, we anticipate that the Y354H mutation would increase the capacity of virions to activate IRF-3.

3.1.2.5. Potential problems and alternative approaches. It is possible that innate immune activation is not due to structural alterations in the outer capsid or core, and therefore would not be identified through these approaches. For example, purified virions contain large quantities of small single-stranded RNA oligonucleotides, which are believed to be products of abortive transcription by the viral polymerases (67, 68) It is not clear if these transcripts would be recognized by RIG-I or Mda-5, but if these oligonucleotides are responsible for IRF-3 activation, we would anticipate that transfected cores would not activate IRF-3, as these transcripts are not present in cores. Additionally, exposure of these transcripts would also not be influenced by capsid stability, and the phenotype should map to the viral polymerase, encoded by the L1 gene segment. This would be indentified by experiments proposed in the next section. It is also possible that the Y354H mutation in σ3 might not exhibit any difference in IRF-3 activation compared to the parental virus. Many other reovirus temperature-sensitive or ethanol resistant mutations have been identified that exhibit alterations in capsid morphology and stability (69-71), and these mutations would

then be examined for their capacity to activate IRF-3. The choice of mutants to test will be informed by the results of the genetic mapping experiments detailed in the next section.

3.1.3 DETERMINANTS OF REOVIRUS ANTAGONISM OF INNATE IMMUNE RESPONSE ACTIVATION.

3.1.3.1. Rationale. Determinants of IFN production in reovirus infected murine cardiac myocytes were mapped to the M1, S2, and L2 genome segments using traditional reassortant genetic methods (24). However, levels of IFN accumulation can be influenced by multiple viral and cellular factors. For example, we recently identified the T1L μ2 protein, encoded by the M1 gene, as an antagonist of IFNAR-dependent signaling (46). Since IFNAR signaling acts as a positive feedback mechanism for IFN production, through the synthesis of IRF-7 (47, 48), disruption of this pathway will limit the total amount of IFN produced despite similar levels of primary IFN induction. Our preliminary data suggests that T1L and T3D differ in their capacity to activate IRF-3 at early times post-infection (Section 1.4.1, Fig. 2), which cannot be attributed to μ2-mediated inhibition of IFNAR signaling. Therefore, genetic determinants that influence the capacity of reovirus to activate IRF-3 remain unknown.

Experiments proposed here will first identify reovirus genes that influence IRF-3 activation using single-gene reassortant viruses, produced using reverse genetics. If differences between T1L and T3D map to structural gene segments, we will then assess differences in capsid stability using the methods described in section 3.1.2. If the differences map to non-structural genes, or if differences in capsid stability do not account for differential IRF-3 activation, we will determine the capacity of ectopically expressed T1L proteins to block IRF-3 activation following T3D infection. These studies complement those proposed in section 3.1.2 and will allow us to identify reovirus structural components that contribute to differences in capsid stability that influence innate immune responses, or to identify novel IFN antagonists.

3.1.3.2. Analysis of T1LxT3D monoreassortant viruses. To identify genetic determinants associated with IRF-3 activation, recombinant T1L and T3D strains will be produced containing a single gene segment derived from T3D or T1L, respectively. We will focus our initial analysis genes previously identified to be associated with IFN induction: M1, S2, and L2. HeLa cells will be infected with these viruses, and IRF-3 activation at 6 h post-infection will be determined as above. Parental viruses rsT3D and rsT1L will be used as positive and negative controls for IRF-3 activation, respectively. If no alterations in IRF-3 activation are observed following infection with these monoreassortant viruses, we will assess the full panel of monoreassortants. These studies will identify gene segments associated with differences in activation of IRF-3 following reovirus infection.

3.1.3.3. Analysis of differences in capsid stability between T1L and T3D. If a structural gene were associated with the capacity of T1L or T3D to activate IRF-3, it would indicate that differences in capsid stability might be responsible for this phenotype. We would then apply the methods described in section 3.1.2 to biochemically analyze these differences. For example, if the S2 gene, encoding core protein σ2, is identified, we will test differences in the capacity of T1L and T3D cores to activate IRF-3, as described in section 3.1.2.2. These experiments will identify differences in exposure of cytoplasmic dsRNA following T1L and T3D infection.

3.1.3.3. Analysis of ectopic expression of T1L proteins. Experiments with single gene reassortants will identify which gene segments are associated with IRF-3 activation, but will not discriminate between T3D gene segments that have agonistic activity (i.e. exert a positive influence on IRF-3 activation) and T1L gene segments that have antagonistic activity (i.e. repress IRF-3 activation). To discriminate between these possibilities, and potentially identify reovirus IFN antagonists, we will ectopically express T1L-encoded proteins in HeLa cells by transient transfection of pCAGGs expression constructs. Following transfection, cells will be infected with rsT3D, and IRF-3 activation will be assessed. An empty pCAGGs construct will be used as a negative control, and T1L protein expression will be confirmed by immunoblot. We used a similar approach to confirm the antagonistic activity of the T1L M1 gene on IFNAR signaling (46). Again, we will focus our initial analysis on genes previously associated with differences in IFN induction (M1, S2, and L2), and proceed to the rest of the T1L genes if those experiments are unsuccessful. If a T1L-derived protein that interferes with IRF-3 activation is identified, these results will be confirmed by generating an rsT3D monoreassortant encoding the T1L gene and assessing the capacity of that virus to activate IRF-3.

3.1.3.4. Potential problems and alternative approaches. It is possible that the capacity of reovirus to activate IRF-3 is not a monogenic trait, and therefore could not be identified by monoreassortant analysis. This is certainly possible if activation of IRF-3 is a function of virion stability and is dependent upon interactions between multiple structural proteins comprising the outer capsid or core, as determined by experiments described in section 3.1.2. To address this, multigene reassortants encoding only the structural genes of T3D or T1L would be produced to exclude a role for the nonstructural genes in these phenotypes. Then, targeted gene substitutions would be made to examine the involvement of either the reovirus outer capsid (derived from the L2, M2, S1, and S4 genes) or core (derived from the L1, L2, L3, M1, and S2 genes). If this approach remains unsuccessful, a traditional reassortant analysis would be performed using a panel of T1LxT3D-derived viruses to statistically identify genes associated with IRF-3 activation. These results will also be used to inform the ectopic expression experiments. It is also possible that transfection efficiency in HeLa cells, which are easily imaged via confocal microscopy, will not be sufficient to elucidate an effect of T1L proteins on IRF-3 activation. In this case, 293T cells, which are readily transfectable but not amenable to precise imaging, will be used, and native PAGE followed by immunoblotting will be used to assess IRF-3 activation.

3.2. SPECIFIC AIM 2. *To identify reovirus strain-specific IFN-dependent and -independent transcriptional networks.*

3.2.1. GENERAL EXPERIMENTAL APPROACH.

Apoptosis induction and IFN production and sensitivity are important determinants of reovirus pathogenesis in the CNS and heart. Transcription factors IRF-3 and NF-κB are required for the induction of both IFN production and apoptosis following reovirus infection (16, 43, 45). However, IFN signaling is not required for reovirus-induced apoptosis induced by reovirus infection (Section 1.4.2, Fig. 3-4), suggesting that the transcriptional networks under IRF-3- and NF-κB-control leading to IFN production and apoptosis are separate and distinct. **Experiments proposed in this specific aim will use microarray analysis to elucidate IFN-dependent and -independent transcriptional networks induced by reovirus infection.** These experiments will be conducted using T1LxT3D M1 monoreassortants. Gene networks induced by these viruses will be identified by microarray. Specific genes identified to be of interest for apoptosis induction will be validated by qPCR and through the use of siRNA knockdown or available null cell lines. These experiments will identify genes and gene networks that are associated with the induction of apoptosis in reovirus-infected cells.

In addition to the biological significance of the questions being addressed, microarray profiling experiments were chosen because of the availability and accessibility of resources for the use of genomics techniques for undergraduate students. The GCAT consortium is a network of educators interested in bringing genomics technology into undergraduate research and education. These efforts will synergize with projects proposed in **Specific Aim 3** to integrate analysis of transcriptional responses to reovirus infection into the Biology curriculum at Colgate University.

3.2.2. CONTRIBUTION OF THE M1 GENE TO REOVIRUS TRANSCRIPTIONAL RESPONSES

3.2.2.1. Rationale. Reovirus T3D is a potent inducer of IFN and apoptosis, whereas T1L is a weak inducer of both phenotypes (24-27). Our preliminary data suggests that these phenotypes are not linked mechanistically (Section 1.4.2). Identification of the reovirus T1L μ2 protein as an antagonist of IFNAR signaling (46), coupled with reverse genetics techniques (51, 52), allows us to functionally decouple cellular transcriptional responses leading to the IFN response from those leading to apoptosis. Using reverse genetics, we generated a panel of 4 viruses, rsT1L, rsT3D, rsT1L-T3DM1, and rsT3D-T1LM1, which will allow us to identify IFN-dependent and -independent gene networks induced by T1L and T3D. We anticipate that rsT3D-T1LM1 should induce genes required for apoptosis induction but not for IFN responses (Table 2). The rsT1L-T3DM1 virus should induce neither transcriptional network, similar to rsT1L, since the T3DM1 gene has not been implicated in apoptosis induction and rsT1L also inhibits IFN-induction upstream from IFNAR signaling (Section 1.4.1). However, this reassortant virus will provide insight into the effects on cellular transcription associated with other functions of μ2 in reovirus replication. For example, since the mechanism of T1L μ2-mediated IFNAR antagonism is not known, μ2 may have pleiotropic effects on other cellular transcriptional pathways, which would be elucidated by comparing genes induced by rsT1L and rsT1L-T3DM1. Experiments proposed here will expand our preliminary

Table 2: Anticipated phenotypes of M1 reassortants

Strain	IFN signaling antagonist	Apoptosis	Other T1L μ2 functions
rsT1L	+	-	+
rsT3D	-	+	-
rsT1L-T3DM1	-	-	-
rsT3D-T1LM1	+	+	+

microarray analysis (Section 1.4.3) to earlier and later time points post-infection and validate identified target genes for their role in reovirus apoptosis.

3.2.2.2. Microarray profiling of cellular transcriptional responses to M1 reassortants. Triplicate wells of HeLa cells will be mock infected or infected with the parental and M1 reassortant viruses, and RNA will be extracted at 0, 6, 12, and 24 h post-infection. cDNA containing a specific capture tag will be synthesized from the RNA using the Array 350 kit (Genisphere). cDNA from each experimental time point will be directly compared with the pooled 0 h cDNA, with reciprocal dye swaps between the triplicate samples. Mixed cDNA will be hybridized to human HEEBO microarray slides, synthesized at Washington University in St. Louis and purchased through the GCAT consortium, and labeled with the 3DNA capture reagent (Genisphere). Labeled microarray slides will then be scanned at one of the GCAT scanning facilities.

3.2.2.3. Microarray data analysis. Images derived from the microarray slides will be gridded and segmented using the MagicTool software package available via GCAT (72). From the resulting expression files, genes significantly up- or down-regulated in each of the triplicate samples will be identified. The compiled gene lists will then be used for bioinformatic analysis, such as heat mapping and gene ontology analysis, using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) package provided by the National Center for Biotechnology Information (NCBI) (50), as performed for our previous microarray analysis (45).

3.2.2.4. Microarray validation. It is anticipated that gene ontology and other bioinformatic analyses will identify genes involved in host cell death responses to reovirus infection. Candidate genes will: i) be upregulated following infection with T3D but not T1L; ii) be IFN-independent; and iii) have previously described functions in apoptotic processes. Alterations in the expression level of several (~4-6) identified candidate genes following reovirus infection will be confirmed by qPCR analysis in independent experiments. To assess the role of these candidate genes in reovirus-induced apoptosis, we will either use null cell lines, if available, or an RNAi approach to knock down expression of the gene(s). The capacity of reovirus to induce apoptosis in wild type or null cell lines will be determined using assays for active caspase-3 or by acridine orange staining, as performed previously (16). If these genes are confirmed to have a functional role in reovirus apoptosis, we will then assess the biochemical mechanism(s) through which they induce cell death in reovirus-infected cells. For example, we recently identified a critical role for Bid in reovirus apoptosis (73). If candidate genes have previously described functions in intrinsic pathways of apoptosis, we will attempt to determine how they interface with Bid-dependent processes in inducing apoptosis following reovirus infection. The overarching goal of these experiments will be to identify potential methods of inhibiting reovirus-induced apoptosis and preventing pathogenesis.

3.2.2.5. Potential problems and alternative approaches. It is possible that since reovirus T1L antagonizes innate immune responses via multiple mechanisms, as indicated by our preliminary data (Section 1.4.2) and reassortant experiments statistically linking the S2 and L2 genes to IFN antagonism (24), substitution of the T1L M1 gene into the T3D backbone may be insufficient to fully decouple the IFN response from the apoptotic response following infection. In this case, we will use information derived from **Specific Aim 1**, to identify other genes associated with IFN antagonism, to generate an “IFN-dead” virus in the pro-apoptotic T3D backbone. The transcriptional networks induced by this virus would then be profiled, as above. If these approaches fail to segregate apoptosis induction from IFN signaling, we will profile changes in gene expression induced by T1L and T3D in IFNAR-deficient MEFs. It is also possible that microarray slides or software provided through the GCAT consortium may not be sufficiently robust to accommodate the level of depth of the proposed experiments. In this case, we would then use commercially available microarrays, such as the GeneChip® Human Gene 1.0 ST Array (Affymetrix), similar to those used previously (45).

3.3. SPECIFIC AIM 3. *To integrate investigative research addressing reovirus innate immune responses into an undergraduate biology curriculum.*

3.3.1. GENERAL APPROACH.

As a faculty member at a small liberal arts college, my primary role is that of an educator of undergraduate students. Much of my work is focused on exposing motivated students to meaningful original research exploring cellular responses to viral infection. To this end, I have designed this proposal to provide opportunities for exposing undergraduate researchers to the mechanisms, both technical and intellectual, of basic viral, immunological, and molecular biological research. Students will be integrated into this project at two levels, each representing a different level of intellectual contribution to the project. First, a laboratory component will be added for students taking the upper-level elective Virology course at Colgate University. This laboratory component will comprise a semester-long project, first learning techniques relevant to the generation and culture of virus stocks, and then analyzing innate immune signaling in response to reovirus. Second, independent research is a required component of the Biology curriculum at Colgate University. All students participate in at least one semester of research, with many conducting research projects for a year or more. This requirement is facilitated by courses termed research tutorials, in which six students engage in independent research projects in the lab of a faculty mentor. **I will use funds from this proposed grant to provide necessary resources for these courses, to increase student research participation during the academic year, to fund summer research positions, and to provide support for student travel to scientific conferences.** These projects will significantly enhance opportunities for research and training for Colgate undergraduates, and accelerate my basic research program into innate immune responses to reovirus infection.

3.3.2. DEVELOPMENT OF A LABORATORY COMPONENT FOR AN UPPER-LEVEL VIROLOGY ELECTIVE.

3.3.2.1. Rationale. Biology 373: Virology is offered every other year as an elective course for ~16 Jr./Sr. level biology and molecular biology majors. The prerequisite for the class is Biology 212: Molecules, Cells, and Genes, an introductory course focused on biochemistry and macromolecules, cell structure and function, molecular biology, and genetics. A required laboratory component for Biol 212 covers basic molecular and cell biological techniques, including microscopy and methods of DNA analysis and cloning. Many students entering Virology will also have taken Biology 333: Microbiology, which includes a required laboratory component focused on advanced microscopy, bacterial culture techniques, and bacterial molecular biology. The lecture portion of Virology introduces basic principles of viral replication, surveys different viral families, and ends with examination of various specialized topics in virology, including vaccinology and virus vectors. This proposal would support development of a laboratory component for the Virology class, as a means of providing hands-on experience in the practice of modern virology research. Reovirus is an ideal model system for this purpose: it is a robust system that can be used by students without extensive technical experience in a manner that is safe and does not require extensive containment precautions. It is amenable for use in teaching many basic virologic techniques, such as plaque titering and virus purification, and can be used to probe interesting experimental questions related to virus pathogenesis, which heightens student interest and enthusiasm. This laboratory will provide initial training for students interested in conducting independent research projects in my research laboratory, as discussed below. Importantly, this lab will also generate virus reagents for use in Specific Aims 1 and 2. In short, this lab course will provide a unique opportunity for undergraduate students to gain experience with modern virology techniques, while facilitating research into innate immune responses to reovirus.

3.3.2.2. Laboratory outline. I envision a semester-long project, with pairs of students working to generate and characterize a reovirus single gene reassortant or other mutant. In the first half of the semester students will learn basic cell and virus culture techniques, while generating, plaque purifying, and confirming their viruses. In the second half of the semester, students will characterize their viruses for their capacity to induce innate immune responses. Characterization will focus on using microscopic and reporter techniques to visualize transcription factor activation (i.e. nuclear localization of IRF-3 by immunofluorescence or activation of luciferase-based IRF-3-, NF-κB-, or ISRE-dependent reporter constructs) in response to virus infection. These assays are straightforward, and I have previously taught undergraduates to use them. All student data will be shared with the instructor and other students via a website database, and used for final analysis by all groups. This will function to emphasize the importance of teamwork and collaboration in scientific investigation. Throughout the semester, students will be

required to engage the primary literature on the subject, culminating with the eventual submission of a primary journal article that includes appropriate introduction, methods, description of results, analysis of observations, figures and legend, and literature cited. Students will be assessed for comprehension of material relating to virus methods and innate immune responses to viruses prior to and after the laboratory. Upon completion of the laboratory, students will also be asked to provide qualitative and quantitative assessments of the laboratory to be used in evaluation of the exercise. These evaluations will be presented for publication in a science education journal such as the *Journal of Microbiology and Biology Education* as well as at the national meeting of the American Society for Virology.

3.3.2.3. Potential problems and alternative approaches. As with most undergraduate laboratories, I anticipate that multiple problems will arise with student projects throughout the course of the semester. This can be particularly troubling in the course of semester-long projects. To prevent technical errors from negatively impacting the lab, I will build redundancy into the projects where possible. For example, multiple groups will attempt to recover the same reassortant, and can share resources if one group's recovery efforts are not successful. I will also have previously-prepared virus stocks for groups to use should individual groups stocks become contaminated. Reserve cell cultures and culture media will also be maintained and provided to students when necessary.

3.3.3. UNDERGRADUATE RESEARCH EXPERIENCES FOR STUDENTS IN INNATE IMMUNE RESPONSES TO REOVIRUS INFECTION.

3.3.3.1. Rationale. It is critical that undergraduate science majors receive the opportunity to perform original research. Although Colgate students are exposed to some original research projects in the laboratory components of courses, it does not always adequately expose students to the excitement and intellectual development provided by engagement in independent research projects. A primary goal of this proposal is to provide resources for undergraduate students actively engaged in independent research projects. The experiments outlined in **Specific Aims 1 and 2** of this proposal will be carried out with the assistance of six to eight highly motivated undergraduate students during the academic year, and two students each summer. The techniques outlined within this proposal are intentionally designed to be accessible to well-prepared undergraduates, and to offer excellent training in cellular, molecular, virologic, immunologic, and bioinformatic techniques and concepts. Indeed, I have had research students employ almost all of the techniques proposed in this study (Section 1.4). Importantly, students engaged in research projects in my lab participate in a weekly lab meeting and journal club to discuss the results of their experiments and facilitate exploration of the relevant primary literature. Although the opportunity has not arisen in the two years in which I have been at Colgate, I also anticipate opportunities for students to present their findings at scientific conferences and participate in publication and grant-writing activities. In the two years I have been at Colgate, I have had 17 undergraduate researchers in my lab, including 4 who performed research for more than one semester and 4 who worked in my lab during the summer. Of these 17 students, 4 have entered graduate programs (including Duke, M.I.T., and the University of Wisconsin), 1 has enrolled in medical school, 4 are technicians in academic research labs, and 5 are still undergraduates at Colgate. I have developed an effective undergraduate research program, and this grant will allow me to maintain the level of undergraduate involvement in my research program I have enjoyed over the past two years.

4. SUMMARY AND FUTURE DIRECTIONS.

Reovirus exhibits strain-specific differences in diseases caused in mammalian hosts. At least part of these differences can be attributed to differences in the activation of innate immune responses leading to IFN induction and apoptosis. The proposed research will identify mechanisms of reovirus innate immune antagonism and define the cellular transcriptional networks responsible for apoptosis induction. As these pathways are likely to be employed by other pathogenic microbes, the proposed experiments might uncover broadly conserved mechanisms of infectious disease pathogenesis. Future studies will focus on the molecular mechanisms used by T1L to antagonize IRF-3 activation, through mutational analysis of the genetic determinants associated with this phenotype identified in **Specific Aim 1.** Additionally, the identification of genes associated with reovirus apoptosis in **Specific Aim 2** will provide a solid foundation for experiments defining the role of these genes in cell death pathways initiated following infection. Significantly, these projects will provide exciting and intellectually challenging opportunities for Colgate's outstanding students to engage in basic virology research as they prepare for careers in biological and biomedical research.

10. VERTEBRATE ANIMALS

No animals will be used for this proposal. All murine embryonic fibroblasts (MEFs) obtained for use in experiments were generated by other laboratories for their own use, and their derivation has been described in published manuscripts. Therefore, this proposal is exempt from Institutional Animal Care and Use Committee (IACUC) review.

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73. [redacted]

DAVIDSON

[redacted]

15 June, 2010

Dear NIAID review panel:

The intent of this letter is to describe the resources of the Genome Consortium for Active Teaching (GCAT) that are available to Geoffrey Holm's current NIH-AREA proposal "Mechanism and functional consequences of reovirus innate immune activation."

GCAT is a global network of faculty members engaged in undergraduate genomics education. The goal of GCAT is to bring functional genomics to the undergraduate curriculum, primarily through student research. GCAT purchases microarray slides from a number of different academic suppliers, and provides them to consortium members for $20 plus shipping. Arrays are available for multiple species, including mouse and human. The arrays are used by undergraduate students for their experiments, and are returned to GCAT to be scanned at one of several scanning facilities for no additional cost. The data are sent via FTP to the researchers for analysis. Prerequisites for the use of GCAT resources are that i) all experiments are performed by undergraduate students and their faculty advisors; ii) all data derived from the experiments are public domain; iii) the faculty member bears the cost of the reagents for the experiments; iv) the faculty member is willing to helping other faculty members by answering questions on the GCAT listserv (see below), when able; v) the faculty member and students complete an assessment survey prior to and after the experiments. A listserv (GCAT-L) connects faculty members who are using, or want to use, genomic methods in their undergraduate courses, and serve as a technical support resource.

GCAT has enjoyed considerable success since its initiation in 2000. At least 9 research articles and 15 educational articles have been published using GCAT resources. GCAT has received funding from the Howard Hughes Medical Institute, the National Science Foundation, and the Waksman Foundation for Microbiology. We are committed to supporting undergraduate research, including projects such as Dr. Holm's.

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Cornell University
College of Veterinary Medicine
Baker Institute for Animal Health

Hungerford Hill Road
Ithaca, New York 14853
t. 607.256.5600
f. 607.256.5608
baker_institute@cornell.edu
http://bakerinstitute.vet.cornell.edu

June 16, 2010

To: NIH Review Board
Cc: Geoff Holm, Ph.D.
Department of Biology
Colgate University
Hamilton, NY 13346

Re: Letter of Support for Dr Holm's NIH-AREA Application, "Mechanism and Functional Consequences of Reovirus Innate Immune Activation".

Dear NIAID review panel:

The purpose of this letter is to indicate my enthusiastic support of Dr. Geoffrey Holm's research program at Colgate University, especially with regard to his current NIH-AREA proposal "Mechanism and functional consequences of reovirus innate immune activation."

Geoff and I have known each other as members of the reovirus research community for five years. I first got to know him as part of a collaboration with his post-doctoral mentor, Dr. Terence Dermody. Since his move to Colgate, we have continued to foster our relationship. I have visited his lab and given a seminar for the Colgate biology department. Geoff has also visited me in Ithaca several times, and has brought his summer research students to my lab to learn some immunofluorescence techniques. He has also participated in the Cornell Virology Program retreat, giving a seminar on his research.

I envision supporting his research program in several ways. First, I will continue to occasionally host Geoff and his research students in my lab, as a means of broadening their access to the reovirus research community. In the same vein, I will facilitate access for Geoff and his students to the Cornell University Program in Virology Seminar Series and Annual Retreat, to further their exposure to current virology research. I will also facilitate access to specialized equipment, such as a cell sorter, that is not available at Colgate. Finally, I will provide other technical support and assistance, when necessary.

I have enjoyed having the opportunity to work with Geoff and to foster interaction between Colgate University and Cornell Program in Virology. I look forward to supporting the research program Geoff has outlined in his NIH-AREA proposal.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No

Tracking Number:GRANT10639260 Funding Opportunity Number:PA-10-070 Received Date:2010-06-24T16:08:55-04:00