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TITLE: New Genetic Tools for Comparative Analysis of Emerging Viruses and Virus-Host Molecular Interactions in Reservoir Hosts versus Spillover Hosts

PRINCIPAL INVESTIGATOR: Welkin Johnson

CONTRACTING ORGANIZATION: Trustees of Boston College
Chestnut Hill, MA 02467

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BOSTON COLLEGE, TRUSTEES OF BOSTON COLLEGE
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14. ABSTRACT Emerging viruses pose significant problems for military personnel living in close quarters, and/or deployed overseas. Significant gaps remain in our understanding of the underlying molecular mechanisms of viral emergence. To fill this gap in knowledge we seek to develop a robust method for identifying the binding partners of small viral proteins implicated in modifying host cell defenses. We are employing a cutting edge genetic system for incorporating synthetic amino-acids at defined positions in proteins within cellular systems; these non-canonical amino-acids (ncAAs) are modified with a side chain that can be induced by UV light to covalently cross-link cellular binding partners. This effectively "tags" the target proteins allowing subsequent identification by mass-spec. We have generated the necessary mutants of two viral proteins, Vpr of HIV-1 and ORF4a of MERS-Coronavirus, and have conducted a pilot mass-spec experiment. All tools and reagents are now in place, and in the next/final period of this 18-month Discovery Award we plan to move forward with mass-spec identification and experimental confirmation of candidate binding partners.

15. SUBJECT TERMS
Vpr, Vpx, ORF4a, HIV, AIDS, MERS-CoV, non-canonical amino-acids, genetic code, emerging viruses, viruses, respiratory virus

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1. **INTRODUCTION:**
2.

Emerging viruses pose significant problems for military personnel living in close quarters, and/or deployed overseas (where exposure to novel viruses often occurs), and are of concern due to the potential for use as bioweapons and bioterrorism agents. While extensive efforts have done into epidemiological surveys and forecasting models, there remains a glaring gap in our understanding of the molecular mechanisms of viral emergence, and in particular why some viruses fail to emerge, others jump into humans and cause disease but fail to spread, and others successfully spread and adapt to humans. This gap in knowledge could be filled by identifying the virus-host molecular interactions that differ between the two host contexts, and by pinpointing genetic differences that drive adaptation of emerging viruses. We are using a cutting-edge approach that allows the modification of viral proteins in such a way that facilitates our ability to "capture" the cellular proteins that are bound or degraded by certain viral proteins, as a first step towards understanding how these viral proteins help emerging viruses adapt to new hosts. Small viral proteins can be modified to incorporate a synthetic amino-acid at specific positions; artificial amino-acids with a cross-linkable side-chain can be used to covalently capture and identify binding partners of these proteins by mass spec. Once identified, appropriate virological assays can be applied to elucidate the contributions of the interactions to viral replication and/or pathogenesis. We are applying this approach to the Vpr protein of HIV-1 and the ORF4a protein of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV); the functions of both proteins are still unclear, and there is good reason to expect that they have novel and as yet unidentified binding partners that may hold the key to understanding their functions. The goal of this Discovery project is to identify candidate binding partners for these proteins as the foundation for future molecular work.

3. **KEYWORDS:**

Vpr, Vpx, ORF4a, HIV, MERS-CoV, non-canonical amino-acids, genetic code, emerging viruses

4. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The goals of the first year of the project were:

1. Identification and mutation of candidate sites in Vpr and ORF4a homologs 1-3 mos. 100%
2. Confirm amber- suppression allows expression and retention of phenotypes and selection of mutant(s) for mass spec experiments 3-6 mos. 50%
3. Mass spec & bioinformatics; selection of hits for further characterization 6-10 mos. 50%
4. Confirmation of hits, mapping of interacting sites, construction and hypothesis testing using single-cycle or replicating HIV/SIV clones 10-16 mos. In progress

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

The focus in the first 12 months has been on creating and validating the suite of reagents and tools necessary to successfully identify binding partners by mass spec. We have chosen specific strains of the viruses based on a survey of the relevant literature, and engineered panels of mutants incorporating the UAG codon in several positions for each. This is necessary, to increase the chances of placing a cross-linkable side-chain in proximity of a binding partner interface, while at the same time minimizing disruption of the interaction. Since the binding partners are unknown (identifying them is the goal), the approach is necessarily empirical. Sites are chosen based on existing structural data and published mutagenesis studies, as well as choosing sites that are predicted to "tolerate" variation and to be exposed on the protein surface. We now have a panel of approzimately a dozen variants for each protein of interest, as well as controls as described in the original proposal, and we have confirmed expression by western blot. As we are close to the goal of conducting mass spec analyses, we also initiated pilot experiments using the wild type proteins extracted under different conditions and analyzed these by mass spec using the same machine and procedures that will be used in the final screen; conveniently, there are known binding partners for HIV Vpr (DCAF1 and UNG) and we were therefore able to validate the mass spec protocol and choice of virus strain because both proteins were re-identified in the screen. In addition, the additional unverified "hits" from this analysis can be compared to the final list, with priority given to those that were identified independently in both screens. When the project is complete, the lists of top candidates will be made publically available (online or in the primary publication).

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the

purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will have a complete set of validated mutants ready for mass-spec screening within the next 4-6 weeks, and will conduct the final mass spec screens in June-July. If we successfully identify one or more candidate binding partners, we will confirm the top hit or hits biochemically and by single-cycle virus infectivity assays during July-September. We have all the necessary assays in place and have used these for many years, so once hits are identified, we will be able to confirm these in short order.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to Report

that

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We were slightly delayed in the validation of the initial constructs, in part because of the efficiency of incorporation of the non-canonical amino-acids and the effect of the incorporation of the ncAA into other cellular proteins. To circumvent these issues, we moved to a system in which the genes of interest, as well as the tRNA synthetases, are co-expressed from the same plasmid. These larger, second generation plasmids were developed in our colleagues lab (Chatterjee lab) at Boston College, and were therefore immediately available for use on this project.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None noted.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None (no human subjects)

Significant changes in use or care of vertebrate animals

None (no vertebrate animals)

Significant changes in use of biohazards and/or select agents

No changes

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

<u>Example:</u>

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

See Next Page for Participants

Name: **Welkin Johnson**
Project Role: PI
Researcher Identifier: ORCID 0000-0001-5991-5414
Nearest person month worked: 1

Contribution to Project: Recruited personnel to the project, oversaw planning and execution of experiments, and assisted with interpretation of results.
Funding Support: This award

Name: **Andrea Kirmaier**
Project Role: Senior Postdoctoral Researcher
Researcher Identifier: ORCID 0000-0001-7206-7640
Nearest person month worked: 10

Contribution to Project: Dr. Kirmaier was responsible for designing HIV and SIV mutant viral proteins, and conducts expression and infection experiments involving primate immunodeficiency viruses and their Vpr proteins (including BSL2+ cell culture and infected cell flow-cytometric analyses).
Funding Support: This award

Name: **Brigitte Lawhorn**
Project Role: Graduate Student
Researcher Identifier:
Nearest person month worked: 7

Contribution to Project: Ms. Lawhorn was responsible for creation of viral protein homolog mutants, and specifically assisted with the production and evaluation of HIV and SIV Vpr mutants.
Funding Support: This award

Name: **Amy Valera**
Project Role: Postdoctoral Research Fellow
Researcher Identifier: ORCID 0000-0001-7897-8541
Nearest person month worked: 2

Contribution to Project: Dr. Valera took over the responsibilities previously performed by Brigitte Lawhorn following her departure from the project.
Funding Support: This award

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report •

8. SPECIAL REPORTING REQUIREMENTS: N/A

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: N/A

9. APPENDICES: *N/A*

New Genetic Tools for Comparative Analysis of Emerging Viruses and Virus-Host Molecular Interactions in Reservoir Hosts versus Spillover Hosts

Grant Log #PR172274

W81XWH1810051

PI: Johnson, Welkin **Org:** BOSTON COLLEGE, TRUSTEES OF BOSTON COLLEGE **Award Amount:** $313,000

Study/Product Aim(s)

- Identify binding partner(s) for HIV-1 Vpr proteins
- Identify binding partners for MERS-CoV ORF4a proteins
- Validate candidate interactions biochemically and in cell culture
- Report/disseminate results

Approach

Candidate binding partners for these small, nonstructural viral proteins will be identified by engineering variants of the proteins to incorporate a synthetic amino-acid (aka a non-canonical Amino-acid or ncAA) at specific sites in or around putative binding sites for the unknown cofactors. The ncAA's have a side-chain that is chemically reactive to UV light, and will form a covalent cross-link with any bound factors, which Will then be visualized by protein blotting and identified by mass spectrometry. Top hits will then be confirmed biochemically and functions revealed using virological methods in cell-culture systems.

Gene name	Peptide counts		
	MERS ORF4a	CTRL 1	CTRL 2
DHX9 - DHX9 ATP-dependent RNA helicase A	27	11	10
TRI25_HUMAN – TRIM25 E3 biquitin/ISG15 ligase TRIM25	24	5	2
DDX1 - DDX1 ATP-dependent RNA helicase DDX1	15	4	4
NKRF - NKRF NF-kappa-B-repressing factor	13	2	0
DSRAD - ADAR dsRNA-specific adenosine deaminase	10	2	1

Selected candidates from a preliminary mass spec screen for binding partners of the MERS Coronavirus ORF4a protein – these candidates were selected from a much longer list for further validation, based on known roles in the innate immune response to virus infections.

Timeline and Cost

Activities	Year 1		
	3-6mo	6-12mo	12-18mo
Subtasks 1.1, 2.1			
Subtasks 1.2, 2.2			
Subtasks 1.3, 2.3			
Subtasks 1.4, 2.4			
Estimated Budget ($K	$240,655		$72,345

Updated: May 1, 2019

NOTE: The two Major Tasks run in parallel and have identical timelines across 18 months. The subtasks are listed together.

Subtasks 1.1, 2.1– design and create mutants ☑
Subtasks 1.2, 2.2 – confirm expression and phenotypes ☐
Subtasks 1.3, 2.3 – mass spec and selection of hits ☐
Subtasks 1.4, 2.4 – confirm hits, cell-culture based testing ☐
Subtasks 1.5, 2.5 – dissemination of results ☐

Comments/Challenges/Issues/Concerns

- This Discovery award is for 18 months total. At this point (12 months) we have met subtasks 1.1/2.1, and are still on target for subtasks 1.3-1.5 and 2.3-2.5. Subtasks 1.2/2.2 are slightly delayed, but will still be completed by the end of the second and final period (n=months 12-18), as described in the RPRR.

Budget Expenditure to Date

Projected Expenditure: $240,655 for first 12 months
Actual Expenditure: $194,554