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TITLE: Therapeutic Targeting of FLCN-Deficient Renal Cancers

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6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goals of this proposal are: 1) to identify the cellular phospho-proteome regulated by FLCN, 2) to discover the mechanisms by which FLCN suppresses the non-canonical translational initiation of a specific subset of mRNAs, and 3) to assign FLCN functions to specific protein domains.

So far we profiled the cellular proteome changes regulated by FLCN in vitro and we gained major insights into the mechanism by which FLCN regulates protein translation.

15. SUBJECT TERMS

Folliculin, GTPase activity, Rab7A, Phosphoproteome, Receptor Tyrosine Kinase activity, Tumor suppressor gene.

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Unclassified	Unclassified	Unclassified	Unclassified	16	

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Folliculin (FLCN) is a tumor suppressor gene linked to the development of renal cell carcinoma (RCC). My laboratory showed before that FLCN acts as a GAP protein for Rab7A and regulates trafficking and therefore activity of EGFR. Our proposal's goal is to show that (1) FLCN regulates not only EGFR, but a panel of cell surface receptor tyrosine kinases in a way similar to EGFR, because the "internalization" of these kinases is a general mechanism of regulation. (2) We showed that FLCN suppresses protein translation and it binds to two translation-promoting factors that are GTPases. We therefore propose to take a system biology approach in order to profile all the kinases that are regulated by FLCN and to evaluate which kinases can be used to target FLCN-driven, rare RCCs. We also propose a series of experiments that will uncover the biochemical details of how FLCN suppresses protein translation. (3) The third goal is to use mutants of FLCN to find out if all these functions depend on different parts of the protein and can be separated from each other. Such a "distribution" of functions (called "domains") will help understand which of these functions are important for tumor suppression.

KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Folliculin, GTPase activity, Rab7A, Phosphoproteome, Receptor Tyrosine Kinase activity, Tumor suppressor gene, Renal Cell Carcinoma

2. **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.

Specific Aim 1: To determine the receptor tyrosine kinases (RTKs) and the cellular phospho-proteome changes regulated by FLCN.

Subtask 1: FLCN-dependent global phosphoproteome changes in cells stimulated with growth factors. Phospho-proteomic and proteomic analysis. Cell lines used: UOK257, FTC-133, infected with wild type FLCN, empty vector control or FLCN mutants.

Subtask 2: FLCN-dependent global phosphoproteome changes in cells stimulated with amino acids. Phospho-proteomic and proteomic analysis. Cell lines used: UOK257, FTC-133, infected with wild type FLCN, empty vector control or FLCN mutants

Specific Aim 2: To fully characterize the biochemical mechanism by which FLCN suppresses initiation of protein translation and identify the subclass of mRNAs regulated by FLCN.

Subtask 1: Establishment of in vitro GAP assay for eIF2gamma and eIF5B.

Subtask 2: Testing in vitro FLCN GAP activity for eIF2gamma and eIF5B.

Subtask 3: Testing tumor associated mutations and post translational FLCN modifications in the GAP assay

Specific Aim 3: To genetically dissect FLCN functions by using a panel of post-translational modification (PTM) and tumor-associated FLCN mutations.

Subtask 1: Generation of tumor-associated FLCN mutants that lead to expression of stable and detectable FLCN mutants

Subtask 2: Generation of single and compound phosphor-mimetic and phospho-inactivating FLCN mutants as well as scanning mutagenesis of FLCN protein.

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.

SPECIFIC AIM 1: Our preliminary data indicated that FLCN regulates several receptor tyrosine kinases (RTKs) through its function as a Rab7A GAP and has a global effect on the cellular phosphoproteome. *We proposed to use proteomic approaches to map the FLCN-regulated phosphoproteomic changes in cells stimulated by growth factors and/or amino acids*. This knowledge will allow us to validate critical nodes of the phosphoproteomic changes as therapeutic targets.

In SOW we proposed to use the FLCN-null UOK257 and FTC-133 cell lines and create isogenic lines in which wild type FLCN was reintroduced. *We completed this goal* and we used FLCN -/- and +/+ cell lines for the experiments described in subtask 1 and subtask 2 of Aim 1. We stimulated the cells lines with amino acids or serum containing growth factors and obtained a phosphoproteome map of the cells. This is achieved through a new phospho-proteomics strategy developed in the Haas laboratory that combines two phospho-peptide fragmentation methods – collision-induced dissociation (CID) and higher-collision-induced dissociation – to increase the sensitivity of multiplexed phospho-proteomics measurements by two-fold (described in our proposal). *We therefore achieved the goal outlined in subtasks 1 and substask 2 in obtaining a map of phosphoproteins regulated by FLCN*.

The attached .xls files (FLCN_proteome and FLCN_phosphoproteome) are the raw data of the proteome changes and phsosphoproteome changes following serum stimulation of three isogenic cell lines: UOK257 FLCN-/- cells, UOK257 FLCN-replete cells, and UOK257 cells infected with the tumor causing FLCN-C9 mutant. The importance of these experiments consists in the discovery that FLCN regulates several receptor tyrosine kinases (RTKs) and their subsequent signaling pathways implicated in tumorigenesis. Future experiments based on this award will ask how to optimally block these pathways with existing drugs, a goal which is beyond the described scope of the award, but very important for development of medical therapies in FLCN-/- renal cell cancers.

To confirm these results in an orthogonal manner and test their conservation through evolution we collaborated with the laboratory of Mo Motamedi, in the MGH Cancer Center. We purified and characterized the Schizosaccharomyces pombe Bhd-Fnp Complex (BFC) and its cellular interactors. We showed that BFC complex physically interacts and regulates the highly conserved peptide transmembrane transporter Ptr2, the phosphoribosylformylglycinamidine synthase Ade3, and the V-ATPase complex. These are novel amino acid-dependent regulators of TORC1. BFC mutants exhibited a slower TORC1 repression and proliferate faster than wild-type cells following amino acid starvation. Therefore, we showed that the mammalian function of FLCN on mTORC1 through regulation of transmembrane RTKs is conserved evolutionarily. This work was published in *iScience* ***(Calvo et al. The fission yeast FLCN/FNIP complex augments TORC1 repression or activation in response to amino acid availability)***

SPECIFIC AIM 2: We showed that FLCN localizes to the polysomes, associates with factors regulating the initiation of protein translation and inhibits eIF2γ and EIF5B complex formation with tRNA-Met_i. The FLCN C-terminal domain, which is deleted by tumor-associated FLCN mutations, is necessary for the interaction of FLCN with eIF2γ and EIF5B. Reintroduction of wild type but not a C-terminus FLCN mutant into FLCN-/- cells results in suppression of serum or amino acid stimulated protein translation, independently of mTORC1 activity. Our data provide insights into a novel mechanism of cell growth restriction by FLCN (*We therefore achieved the goal outlined in Aim 2, subtasks 1, 2 and 3*).

We attach the manuscript "HUMAN FOLLICULIN TUMOR SUPPRESSOR PROTEIN BINDS TO TRANSLATION INITIATION FACTORS eIF2γ AND EIF5B AND SUPPRESSES PROTEIN SYNTHESIS" by Schneider et al, which describes the experiments corresponding to Aim 2A. This manuscript is currently under review.

In the second year of our work we tried hard to establish an in vitro GAP assay for the elongation factors eIF2γ and EIF5B, which are known to undergo secondary modification and be GAP targets. We were **not** able to establish such an assay, despite external collaborations with experts in the field. We will drop now this effort, because, despite being scientifically rigorous work, it may reflect unknown factors not present in the reactions and it detracts efforts from our main goal in analyzing FLCN tagets.

*Work that remains to be done during N*on Cost Extension (NCE)*:*
My lab suffered major drawbacks during COVID epidemic. We closed down for approximately nine months and we had serious attrition of personnel. This is why we asked for a NCE, in order to complete the proposed experiments.

We are now in the process of completing this work. In our proposal we describe how we will identify the RNAs regulated by FLCN (Aim 2B). We have isolated the polysome fractions shown in Figure 2 of the Schneider et al manuscript, and we extracted RNA. We now conduct RNAsequencing of polysomes and compare the RNA profile between FKCN-/- and FLCN reconstituted isogenic cell lines.

This will complete Aim 2B *(Aim 2, subtasks 4 and 5)* in order to discover these RNAs. We regard this necessary to complete the work for Aim 2 and we will report the final results to DOD at the end of the NCE period.

SPECIFIC AIM 3: The goal here is the dissection of FLCN functions by generating a panel of tumor-associated, truncation, phosphor-inactivating and phosphor-mimetic FLCN mutants (Subtasks 1 and 2).
We accomplished the first step of this goal. *We now have generated a panel of phospho-inactivating and phosphomimetic FLCN mutants. We also generated a panel of tumor associated point mutants*.

There raw data about these mutants, their stability and migration are shown in:
Manuscript by Schneider et al, Figures 1F and G (truncation mutants and tumor associated)
Laviolette et al, Figure 1a,1b, and 3a (Phosphomimetic, phosphor-inactivating and tumor associated mutants).

We are in the process of introducing these mutants in FLCN-/- cell lines and to test their ability to suppress the growth of the reconstituted cells as tumors in nude mice. We described this approach (using a patient derived mutant) in our publication: ***Schneider M, Dinkelborg K, Xiao X, Chan-Smutko G, Hruska K, Huang D, Sagar P, Harisinghani M, Iliopoulos O. Early onset renal cell carcinoma in an adolescent girl with germline FLCN exon 5 deletion. Fam Cancer. 2018 Jan;17(1):135-139. doi: 10.1007/s10689-017-0008-8. PMID: 28623476.***

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.

Dr. Iliopoulos trained the following people that so far worked on the FLCN project generating the preliminary results of this proposal, the data included in the manuscripts currently submitted for publication and the data included in the current progress report.
Laura Laviolette, PhD. Obtained her PhD from University of Vancouver in Canada, completed her post-doctoral training with Dr. Iliopoulos, worked on FLCN and she is now an Associate Director, heading the Immuno-Oncology R&D, in Dragonfly Therapeutics, Inc.
Meike Schneider, MD completed her post-doctoral training with Dr. Iliopoulos after graduating from the Department of Urology, Medical Center Johannes Gutenberg University, Mainz, Germany and she is now a senior scientist at Bayer Oncology. She is the lead author in the paper describing the effect of FLCN on protein translation.
Katia Dinkelborg, MD completed her Diploma Thesis with Dr. Iliopoulos, graduated from University of Hannover Medical School in Germany and she is now working as a research fellow in the University of Hannover Department of Hepatology.
Ravi Sundaram, BS, obtained his Bachelor of Sciences from Northeastern University, Boston, MA and worked as a Research Technician for 3 years at the Iliopoulos Lab. He is now enrolled in the University of Sydney Medical School, in Sydney, Australia.
D**ongkook Min, PhD,** is a senior post-doctoral fellow recruited to the lab in January 2022, in order to complete the experiments remaining above and expand the FLCN project observations to deeper analysis.

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.

The main avenue of data dissemination is publication of our data. In addition, we generated several collaborations, attracting colleagues to study the basic mechanism of tumor suppression by FLCN (as evidenced by our publication record) and presented the data in local national and international scientific meetings.

The following manuscripts are submitted for publication or published. These manuscripts were supported by the DOD award at hand.

1) **HUMAN FOLLICULIN TUMOR SUPPRESSOR PROTEIN BINDS TO TRANSLATION INITIATION FACTORS eIF2□□AND EIF5B AND SUPPRESSES PROTEIN SYNTHESIS** (Meike Schneider, Katja Dinkelbor, Syed I.A. Bukhari, Samuel S Truesdell, Vera A. Pisareva, Andrey V. Pisarev, Shobha Vasudevan and Othon Iliopoulos). *UNDER REVIEW*

2) **The fission yeast FLCN/FNIP complex augments TORC1 repression or activation in response to amino acid availability**
(Isabel A. Calvo, Shalini Sharma, Joao A. Paulo, Alexander Gulka, Andras Boeszoermenyi, Jingyu Zhang, Jose M. Lombana, Christina M. Palmieri1, Laura A. Laviolette, Haribabu Arthanari, Steven P. Gygi, Othon Iliopoulos and Mo Motamedi). iScience. 2021 Nov 19; 24(11): 103338.

3) **Genetic risk assessment for hereditary renal cell carcinoma: Clinical consensus statement.** Bratslavsky G, Mendhiratta N, Daneshvar M, Brugarolas J, Ball MW, Metwalli A, Nathanson KL, Pierorazio PM, Boris RS, Singer EA, Carlo MI, Daly MB, Henske EP, Hyatt C, Middleton L, Morris G, Jeong A, Narayan V, Rathmell WK, Vaishampayan U, Lee BH, Battle D, Hall MJ, Hafez K, Jewett MAS, Karamboulas C, Pal SK, Hakimi AA, Kutikov A, Iliopoulos O, Linehan WM, Jonasch E, Srinivasan R, Shuch B. Cancer. 2021 Nov 1;127(21):3957-3966. doi: 10.1002/cncr.33679. PMID: 34343338; PMCID: PMC8711633.

4) **Seventh BHD international symposium: recent scientific and clinical advancement.** Woodford MR, Andreou A, Baba M, van de Beek I, Di Malta C, Glykofridis I, Grimes H, Henske EP, Iliopoulos O, Kurihara M, Lazor R, Linehan WM, Matsumoto K, Marciniak SJ, Namba Y, Pause A, Rajan N, Ray A, Schmidt LS, Shi W, Steinlein OK, Thierauf J, Zoncu R, Webb A, Mollapour M.. Oncotarget. 2022;13:173-81. Epub 20220120. doi: 10.18632/oncotarget.28176. PubMed PMID: 35070081; PMCID: PMC8780807.

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

Within the remaining time of NCE we plan:

1) To identify the mRNAs regulated by FLCN, as described in our proposal (Aim 2, subtasks 4 and 5).
2) To complete testing the effect of FLCN secondary modifications in the ability of FLCN to suppress tumor formation in the xenogrtaft tumor suppressor assay (Aim 3, subtask 3).

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).

We are dissecting the function of FLCN tumor suppressor gene. Our goal is to discover what are the critical biochemical events that are deregulated in cells when FLCN is inactivated. To this end we are linking genetic analysis of FLCN to the biochemical events that are regulated by this tumor suppressor gene, namely changes in the cellular phosphoproteome and in protein translation.

Our work so far provided important insights in the function of FLCN, that clearly influence the field.

The main achievement is that we showed that FLCN targets specific proteins (Rabs) and through them regulates the ACTIVITY of MANY transmembrane receptors that sense growth factors and extracellular nutrients. FLCN tempers the response of the cells to the environment. It provides a "break" for the growth signals to the cells by attenuating how the cells sense the "grow" extracellular signal. FLCN-negative cells "over-respond" to a given "grow" signal and turn malignant.

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.

Our work highlighted the role of Rab7 as a regulator of growth factor and nutrient sensing transmembrane receptors. We therefore influenced the field and the work others.
As an example, colleagues in our immediate environment (Andi McClathey Lab) expanded this observation to their own work. (Chiasson-MacKenzie C, Morris ZS, Liu CH, Bradford WB, Koorman T, McClatchey AI. Merlin/ERM proteins regulate growth factor-induced macropinocytosis and receptor recycling by organizing the plasma membrane-cytoskeleton interface. Genes Dev. 32(17-18): 1201-14, 2018)

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:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

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.

As it is well known this has been a year of hardship for all. Our laboratory was closed for 4 months due to COVID pandemic. The PI was recruited to provide clinical care of COVID patients. In addition he was infected by COVID19 and had a prolonged course of illness. These issues were <u>*recently*</u> resolved and we therefore hope to move forward without any further obstacles. We recently recruited a senior post doctoral fellow (Dr. Min) to complete th remaining experiments described in the FLCN proposal.

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.

Nothing to Report

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 expected

Significant changes in use or care of vertebrate animals

None expected

Significant changes in use of biohazards and/or select agents

None expected

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.*

Genetic risk assessment for hereditary renal cell carcinoma: Clinical consensus statement. Bratslavsky G, Mendhiratta N, Daneshvar M, Brugarolas J, Ball MW, Metwalli A, Nathanson KL, Pierorazio PM, Boris RS, Singer EA, Carlo MI, Daly MB, Henske EP, Hyatt C, Middleton L, Morris G, Jeong A, Narayan V, Rathmell WK, Vaishampayan U, Lee BH, Battle D, Hall MJ, Hafez K, Jewett MAS, Karamboulas C, Pal SK, Hakimi AA, Kutikov A, Iliopoulos O, Linehan WM, Jonasch E, Srinivasan R, Shuch B. Cancer. 2021 Nov 1;127(21):3957-3966. doi: 10.1002/cncr.33679. PMID: 34343338; PMCID: PMC8711633.

Seventh BHD international symposium: recent scientific and clinical advancement. Woodford MR, Andreou A, Baba M, van de Beek I, Di Malta C, Glykofridis I, Grimes H, Henske EP, Iliopoulos O, Kurihara M, Lazor R, Linehan WM, Matsumoto K, Marciniak SJ, Namba Y, Pause A, Rajan N, Ray A, Schmidt LS, Shi W, Steinlein OK, Thierauf J, Zoncu R, Webb A, Mollapour M.. Oncotarget. 2022;13:173-81. Epub 20220120. doi: 10.18632/oncotarget.28176. PubMed PMID: 35070081; PMCID: PMC8780807.

- **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.

MYROVLITIS TRUST
https://myrovlytistrust.org/conference-report-from-the-7th-international-bhd-symposium-october-2021/
This is a Birt-Hogg-Dube (BHD) patient advocate organization. They update the list of basic scientific and clinical publications in the field of FLCN and BHD disease.
Our work was presented in the 7th International BHD Conference (2021) and included in the conference paper.

- **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".

Example:

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.)*

Othon Iliopoulos
PD/PI
1.8 CM
Dr. Iliopoulos is the overall PI of the Project, overseeing the experiments

Yun Liao
Post Doc
12 CM
Post-doctoral fellow who worked in the project pre-pandemic.

Tupa Basuroy
Post Doc
12 CM
She is a post doctoral fellow who worked on the project, post pandemic, until Dr Min was hired to continue the work.

Wilhelm Haas
Co-Investigator
1.2 CM
He is a co-investigator who conducted the proteomic analysis described in Aim 1, collaborating with Dr. Iliopoulos

Shobha Vasudevan
Co-Investigator
0.6 CM
She is a co-investigator in the project, collaborating with Dr Iliopoulos in RNA polysome

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

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*