SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **11/01/2010**

Deirdre Joy
[redacted]

Application Number: **1 R21 AI094129-01**

Principal Investigators (Listed Alphabetically):
MYLER, PETER JOHN PHD (Contact)
PARSONS, MARILYN PHD

Applicant Organization: SEATTLE BIOMEDICAL RESEARCH INSTITUTE

Review Group: **PTHE**
Pathogenic Eukaryotes Study Section

Meeting Date: **10/14/2010**
Council: **JAN 2011**
Requested Start: **04/01/2011**

RFA/PA: **PA10-069**
PCC: **M93**

Project Title: **Ribosome profiling of Trypanosoma brucei**

SRG Action: **Impact/Priority Score: 10**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**

Project Year	Direct Costs Requested	Estimated Total Cost
1	[redacted]	[redacted]
2		
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R21AI094129-01 MYLER, PETER

RESUME AND SUMMARY OF DISCUSSION: This application seeks to use ribosome profiling technology to identify genes under translational control in the major developmental stages of *Trypanosoma brucei*, the causative agent of African trypanosomiasis. If successful, this work will have a huge impact in our understanding of transcription and post-transcription regulation in a medically important protozoan and provide an important resource for the community working on cell and molecular biology of trypanosomatids. Additional strengths are the track record of productivity of the investigator and co-investigator, which combines achievements in the field of molecular biology of kinetoplastid parasites and genomics, and the clever, cutting edge, and innovative approaches. Overall, this almost flawless and exciting application raises very high enthusiasm.

DESCRIPTION (provided by applicant): *Trypanosoma brucei*, the causative agent of African trypanosomiasis ("sleeping sickness"), causes more than 50,000 deaths annually. Related trypanosomatid pathogens, including *Trypanosoma cruzi* (the causative agent of Chagas' disease) and numerous *Leishmania* species (which cause a diverse spectrum of visceral, mucocutaneous, and cutaneous disease), cause even more morbidity and mortality worldwide. Each of these parasites undergoes a complex developmental cycle, alternating between mammalian and insect hosts, as well as proliferating and non-proliferating stages. Exactly how trypanosomatid gene expression gives rise to the different phenotypes at each stage is currently not well understood, but the relative contribution of gene-specific transcriptional control is low. Differences in post-transcriptional mRNA processing and stability undoubtedly play major roles but the poor correlation between mRNA and protein abundance during parasite development indicates that translational and/or post-translational controls are also important. This project seeks to globally and quantitatively assess the rate at which each mRNA is actively translated at any particular time by applying a recently-described technology that couples the ability to isolate the specific "footprints" of mRNAs that are occupied by ribosomes (an indicator of translation) with the depth and breadth of next generation sequencing. Aim 1 will establish the ribosome protection technology in *T. brucei*, using readily cultured non-pathogenic insect stage forms. It will optimize conditions for nuclease treatment to preserve mRNA fragments protected by ribosomes and for the generation of unbiased libraries from the RNA samples for next generation sequencing. It will also include maturation of the bioinformatics pipeline to analyze resulting sequence data. Aim 2 will expand into the pathogenic, mammalian stages of the parasite, and identify genes that are regulated at the level of translation during *T. brucei* development in infective as compared to non-infective forms. The proposed work will provide an important new tool for studying trypanosomatid gene expression, yielding a comprehensive view of the role of translational control in *T. brucei* and clues to it mechanisms, as well as new information on the extent of translation of individual gene products, such as potential drug targets. In addition, it should resolve the current debate over the function of the numerous recently identified RNAs that contain only short open-reading frames, and has the potential to identify non-canonical protein-coding open-reading frames, thus significantly enhancing the ongoing genome annotation.

PROJECT NARRATIVE: The parasite *Trypanosoma brucei* causes fatal human African trypanosomiasis (sleeping sickness) and drugs to treat the disease are toxic and facing resistance. Generating new drugs requires knowledge of which proteins are expressed in the disease-causing stages of parasite development. This project will apply a new technology to measure the initial steps of protein synthesis for all genes in the infective as compared to non-infective stages, thereby providing new information on candidate drug targets.

CRITIQUE 1:

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

Approach: 2
Environment: 1

Overall Impact: An excellent application by an established investigator with a long track record of productivity in both the trypanosome field and the field of genomics. The application is nicely written and aims at applying the recently published technique of ribosome profiling to the study of developmental gene control between different stages of *T. brucei*. The application of ribosome profiling coupled with next generation sequence would be (to my knowledge) a first on the trypanosome field. There is no doubt that the proposed research will finally reconcile the well established differences between the transcriptome and proteome of this organism, where it is now widely accepted that the bulk of the control of gene expression occurs at some step after transcription.

1. Significance:

Strengths

- The approach will no doubt reveal in a gene specific manner what genes are controlled postranscriptionally vs. those whose expression is controlled postranslationally (for which little is known in trypanosomes)

Weaknesses

- It is not clear how significant is to differentiate between stumpy and slender forms given the complications of separating the two forms (see below). This, however, is not a major weakness.

2. Investigator(s):

Strengths

- Very productive investigator well versed in the implementation of a wide array of genomic approaches for the study of trypanosomes
- Excellent collaboration with Dr. Parsons.

Weaknesses

- None.

3. Innovation:

Strengths

- The application of the ribosome profiling technique/deep sequencing is a first for the field.
- Clever and cutting edge.

Weaknesses

- No significant weaknesses

4. Approach:

Strengths

- Well developed rationale
- Excellent discussion of alternative approaches

Weaknesses

- The only weakness is the difficulty of obtaining sufficiently pure stumpy vs. slender forms. However, either way even the simple comparison of PCF to BSF is exciting enough.

5. Environment:

Strengths

- Excellent

Weaknesses

- None.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

Biohazards:

Acceptable

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

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

Overall Impact: The proposed work will examine the ribosome loading on mRNAs in the major developmental stages of the important parasite species *Trypanosoma brucei*. In addition to the medical importance of the system and related organisms, the molecular biology is highly unusual since post-transcriptional gene control is the “rule” in these organisms. The application is by an established pair of investigators with a very strong record of productivity, including in areas involving very large datasets (e.g., genome sequencing). The plan is very clearly written and involves a cutting edge approach that is perfectly suited to the task. A successful outcome will have a very high impact for many investigators in the future. A letter of support from the technique’s developer completes a near flawless application that is perfectly suited to the R21 funding mechanism.

1. Significance:

Strengths

- African trypanosomes are important parasites in their own right as well as being representative of a class of organisms that includes many other pathogens
- Polycistronic transcription and post-transcriptional regulation occur in kinetoplastid organisms to a degree seen in no other system; hence they are perfect places to learn new things about how such control is affected, in general.
- The work will produce genome-wide data that will be of enormous interest to all investigators working on the cell and molecular biology of these parasites.

Weaknesses

- None

2. Investigator(s):

Strengths

- Drs. Myler and Parsons have over 50 years experience between them working on the cell and molecular biology of kinetoplastid parasites, often in very productive collaboration
- They have individually been very productive and performed work at the cutting edge of the field.
- They have collaborated with each other on numerous projects with great success
- Myler has extensive experience leading large, genome-wide efforts (e.g., genome sequencing) that involve exactly the skills needed here, both in terms of personnel and information management (Dr. Myler directs the bioinformatic facility at his institution).
- The method to be used was developed by Dr. Ingolia who has already provided a detailed protocol to the investigators and provides an appropriate letter of support.

Weaknesses

- None

3. Innovation:

Strengths

- This work represents the application of a cutting edge approach to a group of organisms that have not previously been the object of such global analyses.

Weaknesses

- The method was not developed by the investigators and has already been used in other systems (e.g., yeast).

4. Approach:

Strengths

- The protocol is clearly described and follows an entirely logical plan
- Inclusion of the three major life stages will make for extremely interesting comparisons
- The potential to discover the role of some tantalizing, novel ORFs (that are extremely small and of unknown function) is well described
- Enough examples of transcripts that are strongly regulated post-transcriptionally already exist making validation of the data easy to do at an early stage in the experiments

- Excellent bioinformatic analysis

Weaknesses

- None

5. Environment:

Strengths

- Seattle BioMed has a long history of continuous productivity in the field
- The infrastructure necessary to do the work has been well established as a consequence of other genome-wide efforts carried out there
- There are few other places with the track record and resources to make a compelling case for successful execution of this cutting-edge project

Weaknesses

- None

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

Biohazards:

Acceptable

Resource Sharing Plans:

Unacceptable

- The plans to share the data are incomplete in that it is not stated within what timeframe the data will be made publicly available. Please be specific.

Budget and Period of Support:

Recommend as Requested

Additional Comments to Applicant (Optional):

- A great use of R21 funds! A fantastic scenario would have the investigators comparing profiles from different places in the polysome profiles (e.g., from polysomes consisting of 1, 2, 3, etc. ribosomes) but I accept that this would be technically much more challenging and financially take the work outside the scope of a R21.

CRITIQUE 3:

Significance: 1
Investigator(s): 2

Innovation: 3
Approach: 1
Environment: 1

Overall Impact: This is a very strong application that seeks to define the mRNAs that are undergoing translation in *T. brucei*. The strengths are that the investigator and co-investigator are very versed in large scale genomic analysis, have choosen the correct strain (927) that is pleomorphic, have developed a cleaver way to derive both control and polysome associated mRNAs and will likely uncover a meaningful translational profile for protein synthesizing mRNA in trypanosomes. The weaknesses are that the group does not outline the controls they will use and the 'standard' mRNAs they expect to find. For example, can they use the leishmania data they note that they have already to detect highly regulate proteins that are regulated by efficient translation and not simply mRNA abundance? Thus, the major problem is that there needs to be some validation that the undertaken protocol is working. The group is very strong and the collaboration between Dr. Parsons and Myler will yield useful information.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMAL (Resume): ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html.
The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.