| | | |
|---|---|---|
| PI: **Myler, Peter John** | Title: Ribosome profiling of Trypanosoma brucei | |
| Received: 06/15/2010 | FOA: PA10-069 | Council: 01/2011 |
| Competition ID: ADOBE-FORMS-B | FOA Title: NIH EXPLORATORY DEVELOPMENTAL RESEARCH GRANT PROGRAM (PARENT R21) | |
| **1 R21 AI094129-01** | Dual: | Accession Number: 3307346 |
| IPF: 1116301 | Organization: SEATTLE BIOMEDICAL RESEARCH INSTITUTE | |
| Former Number: | Department: | |
| IRG/SRG: PTHE | AIDS: N | Expedited: N |
| Subtotal Direct Costs<br>(excludes consortium F&A)<br>Year 1: [redacted]<br>Year 2: [redacted] | Animals: Y<br>Humans: N<br>Clinical Trial: N<br>Current HS Code: 10<br>HESC: N | New Investigator: N<br>Early Stage Investigator: N |
| | | |
| *Senior/Key Personnel:* | *Organization:* | *Role Category:* |
| Peter Myler PhD | Seattle Biomedical Research Institute | PD/PI |
| Marilyn Parsons PhD | Seattle Biomedical Research Institute | MPI |

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

**2. DATE SUBMITTED** 06/15/2010

**Applicant Identifier**

**4. a. Federal Identifier**

**b. Agency Routing Identifier**

**5. APPLICANT INFORMATION**

*** Organizational DUNS:** [redacted]

* Legal Name: Seattle Biomedical Research Institute
Department:
Division:
* Street1: 307 Westlake Ave N, Suite 500
Street2:
* City: Seattle
County / Parish: King
* State: WA: Washington
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 98109-5219

Person to be contacted on matters involving this application
Prefix:
* First Name: Jennifer
Middle Name:
* Last Name: Dodson
Suffix:
* Phone Number: [redacted]
Fax Number: [redacted]
Email: [redacted]

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

**7. * TYPE OF APPLICANT:** M: Nonprofit with 501C3 IRS Status (Other than 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:**
Ribosome profiling of Trypanosoma brucei

**12. PROPOSED PROJECT:**
* Start Date: 04/01/2011
* Ending Date: 03/31/2013

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT** WA-007

**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**
Prefix:
* First Name: Peter
Middle Name: John
* Last Name: Myler
Suffix: PhD
Position/Title: Member
* Organization Name: Seattle Biomedical Research Institute
Department:
Division:
* Street1: 307 Westlake Ave N
Street2: Suite 500
* City: Seattle
County / Parish: King
* State: WA: Washington
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 98109-5219
* 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: * First Name: Jennifer Middle Name:

* Last Name: Dodson Suffix:

* Position/Title: Manager, Grants and Contracts

* Organization: Seattle Biomedical Research Institute

Department: Division:

* Street1: 307 Westlake Ave N, Suite 500

Street2:

* City: Seattle County / Parish: King

* State: WA: Washington Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 98109-5219

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

* Email: [redacted]

*** Signature of Authorized Representative**: Jennifer Dodson

*** Date Signed**: 06/15/2010

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

# 424 R&R and PHS-398 Specific
# Table Of Contents

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 tribal government, academia, or other type of organization.

Organization Name: Seattle Biomedical Research Institute

DUNS Number: [redacted]

* Street1: 307 Westlake Ave N, Suite 500

Street2:

* City: Seattle County: King

* State: WA: Washington

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 98109-5219 * Project/ Performance Site Congressional District: WA-007

**Project/Performance Site Location** 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal 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

# 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 [redacted]

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** Abstract1003112209.pdf Add Attachment Delete Attachment View Attachment

**8. * Project Narrative** ProjectNarrative1003112213.pdf Add Attachment Delete Attachment View Attachment

**9. Bibliography & References Cited** Bibliography_and_References_Cited1003 Add Attachment Delete Attachment View Attachment

**10. Facilities & Other Resources** Facilities_and_Resources1003112138.pd Add Attachment Delete Attachment View Attachment

**11. Equipment** Equipment1003112139.pdf Add Attachment Delete Attachment View Attachment

**12. Other Attachments** Add Attachments Delete Attachments View Attachments ☐

## Project Summary/Abstract

*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.

**FACILITIES & OTHER RESOURCES – Seattle Biomedical Research Institute**

Environment: The facility, built in 2005, is designed for molecular biological, immunological and biochemical research into globally important pathogens and their interactions with the host. Space is assigned according to the needs of the project and laboratory staffing, ensuring adequate space for funded projects. The use of shared equipment allows cost savings for individual grants and investigators, and the access to specialized cores enables investigator use of the latest technologies in a cost- and personnel-effective manner. The entire laboratory area is BSL2 (or higher) containment, including the animal facilities. An active contingent of parasite biologists studying molecular and cellular processes important in trypanosomatid and apicomplexan diseases makes Seattle BioMed a unique environment for conducting this project. The Trypanosomatid Pathogens group (Parsons, Myler, and Stuart labs) has strong interactions at the staff through Principal Investigator level, enhanced by biweekly literature discussions, and monthly senior staff programmatic meetings. Additionally, Seattle BioMed has initiated bimonthly "ongoing research" discussions on trypanosomatid pathogens with other researchers in Seattle, including those at the University of Washington (currently Hol, Gelb, Van Voorhis, and Buckner).

Laboratory: This project will be conducted within the 112,000 $ft^2$ laboratory and office complex of Seattle BioMed. The space contains seventeen BSL2 interconnecting laboratory areas and centralized facilities. The Parsons laboratory contains a BSL2 tissue culture suite and laboratory benches, which are assigned on the basis of the number of laboratory staff per Seattle BioMed policy. Staff have access to shared facilities including radioisotope, equipment, preparation and dark rooms, as well as a glasswash and sterilization room. Seattle BioMed Core Facilities are made up of 7 state-of-the-art technology centers: DNA Sequencing, Proteomics, Flow Cytometry, Imaging, Protein Production, Bioinformatics and Insectary. Each Core is supervised by a Ph.D-level Scientific Advisor who is available for consultation on experimental design and instrument capabilities. Sophisticated instruments are operated by dedicated staff that possesses the appropriate training and expertise. Core services are charged on a per-sample or hourly basis depending on the service used. Core oversight is conducted by a Steering Committee consisting of PIs and representatives of the administrative and scientific staff. Relevant to this project are:

The DNA Sequencing Core provides routine DNA sequencing and genotyping services including performing sequencing reactions, as well as library construction, DNA isolation and template preparation, primer design, real time PCR, and DNA sequence assembly. The facility is staffed with a Core Manager and technician. Next generation sequencing is not performed in this core, but is purchased on a fee for service basis.

The Bioinformatics Core provides Seattle BioMed researchers access to state-of-the-art infrastructure (hardware and software) and personnel in order to track, store, manipulate, and analyze the biological data needed for hypothesis generation and testing. Servers host a variety of genome-scale software, including sequence alignment and assembly, gene prediction and annotation, local BLAST service (using specialized databases), a client/server-based desktop sequence analysis package, microarray data analysis and statistical packages, and a proteomics analysis pipeline, as well as custom-designed project-specific scripts, software and GUIs. The facility is staffed by a PhD level Core Manager, Database specialist, Bioinformatics Software specialist and Bioinformatics programmer, as well as a Systems Administrator. The staff works with personnel from Seattle BioMed research programs and other Cores to provide support for project-specific data and process management databases, data manipulation and analysis, as well as software training and consultation.

Clinical: not applicable

Animal: This project will use the Seattle BioMed Animal Biosafety Level-2 facility, in which infections with trypanosomes are routinely conducted. The facility contains six animal holding rooms (rodents only), two procedure rooms, a cleaning room, and two storage rooms for a total of ~2,900 square feet. Included in this space are three Specific Pathogen-Free rooms protected by a barrier and a BioBubble air handling system. The BSL-2 animal facility also contains facilities for mouse perfusion under terminal anesthesia, and for survival microsurgery. The facility is isolated and independently ventilated. A second facility is classified and operated as an Animal Biosafety Level-3 facility and will not be used by this project. Animal health is monitored by a veterinarian and makes use of sentinels. Seattle BioMed has an active Animal Welfare assurance on file with OLAW (A3640-01).

Office: The PIs have 140 $ft^2$ offices near the laboratories. Additional office space is available for students, postdocs, and senior staff.

Library: Professional library services at Seattle BioMed include mediated literature searching in scholarly research databases, citation analyses, collaboration portal support, and curation of an institutional repository. Librarian consultation topics include bibliographic citation management software, MEDLINE, NIH Public Access Policy compliance, copyright, social media tools, and individual information management. Scientists have full access to web-based knowledge resources at Seattle BioMed and University of Washington including current journals subscriptions and reference texts. Documents not available online may be requested for delivery via Seattle BioMed's document delivery services. Seattle BioMed is a full member of the National Network of Libraries of Medicine.

IT Servers: The Seattle BioMed network is linked to the Internet via a 20Mbps fiber optic connection providing access to resources at the University of Washington (with which Seattle BioMed is affiliated) and other collaborating sites. The Institute maintains a secure "trusted partners" network to facilitate data exchange with collaborating sites worldwide. The scientific desktop network consists of 200 PC and Macintosh computers linked via a 1 Gbps Local Area Network (LAN). Shared peripheral devices include network storage, color laser printers, high-resolution scanners and multi-media production equipment. Centralized software includes packages for DNA sequence analysis, statistics, proteomics analysis, microscopic image management, laboratory information management systems (LIMS), grants management, word processing and technical graphics. Seattle BioMed's professional IT staff maintains the information technology infrastructure.

Other: An active Environmental Health and Safety group provides ongoing safety training for chemical, radioisotope and biological hazards. All staff working with *T. brucei* receive biosafety training by Seattle BioMed and in lab training from experienced staff. All laboratory space is biosafety level 2.

**EQUIPMENT – Seattle Biomedical Research Institute**

The Parsons and Myler laboratories have standard molecular and cellular biology equipment such as biosafety cabinets, incubators, water baths, PCR machines, electrophoresis equipment, refrigerators, freezers (-20°C and -80°C), liquid nitrogen storage tanks, and shakers. A density gradient fractionation system is available. In addition, Seattle BioMed maintains shared equipment that is available to all labs, including ultracentrifuges, high-speed refrigerated floor centrifuges, fluorescence microscopes, UV-VIS spectrophotometers, absorbance and fluorescence plate readers, a bioanalyzer, liquid scintillation counters, an x-ray irradiator by RadSource (RS2000), and gel documentation systems. This equipment is overseen by the Shared Equipment Manager. Seattle BioMed also has a wide variety of standard scientific equipment for general use, including fume hoods, cold rooms, water purification system, autoclaves, balances, ovens, pH meters, film processor, and other small equipment. This equipment is overseen by the Facilities Manager.

Core equipment is located in the Seattle BioMed Building in rooms adjacent to the general lab space. Sophisticated instruments are operated by dedicated staff that possesses the appropriate training and expertise. Core services are charged on a per-sample or hourly basis depending on the service used. Scheduling of many instruments is accomplished *via* Microsoft Outlook calendars. The equipment is overseen by the Core Manager for each facility. Equipment within cores relevant to this project include a high-throughput Applied Biosystems 3730XL automated 96-capillary DNA Analyzer for routine sequencing and genotyping applications and a 7500 Fast Real Time PCR instrument for measuring gene expression within the the DNA Sequencing Core, and several multi-node Linux clusters with 64-bit processors (and 2-8 GB of RAM per node) for high-capacity (multi-Gigabyte) computation, Windows-based SQL Servers for database management, and Terabyte-scale storage capacity on the Seattle BioMed Storage Area Network, all linked *via* a gigabit-speed TCP/IP network backbone with a fully fault-tolerant architecture that are managed by IT in collaboration with the Bioinformatics Core.

Within Seattle, the University of Washington, Fred Hutchison Cancer Research Center, and Institute for Systems Biology provide access to numerous facilities and equipment on a collaborative or fee-for-service basis. This includes an Illumina Genome Analyzer IIx sequencer at the High Throughput Genome Sequencing Center in the Department of Genome Sciences at the University of Washington.

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

# RESEARCH & RELATED Senior/Key Person Profile (Expanded)

**PROFILE - Project Director/Principal Investigator**

Prefix: | * First Name: Peter | Middle Name: John
* Last Name: Myler | Suffix: PhD
Position/Title: Member | Department:
Organization Name: Seattle Biomedical Research Institute | Division:
* Street1: 307 Westlake Ave N
Street2: Suite 500
* City: Seattle | County/ Parish: King
* State: WA: Washington | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 98109-5219
* 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:** PhD
**Degree Year:** 1982

***Attach Biographical Sketch** Biosketch_Myler1003112199.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: Marilyn | Middle Name:
* Last Name: Parsons | Suffix: PhD
Position/Title: Director of Scientific Operations | Department: N/A
Organization Name: Seattle Biomedical Research Institute | Division: N/A
* Street1: 307 Westlake Ave N, Suite 500
Street2:
* City: Seattle | County/ Parish: King
* State: WA: Washington | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 98109-5219
* 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:** PhD
**Degree Year:** 1979

***Attach Biographical Sketch** Parsons_Biosketch1003112200.p | Add Attachment | Delete Attachment | View Attachment
**Attach Current & Pending Support** | Add Attachment | Delete Attachment | View Attachment

## 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 |
|---|---|
| Peter J. Myler, Ph.D. | Full Member |
| 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 |
|---|---|---|---|
| University of Queensland, Brisbane, QLD, Australia | B.Sc.(Hons) | 1978 | Biochemistry |
| University of Queensland, Brisbane, QLD, Australia | Ph.D. | 1979-1982 | Biochemistry |
| Issaquah Health Research Institute | Post-doc | 1982-1983 | Molecular Parasitology |
| Washington State University | Post-doc | 1984-1985 | Molecular Parasitology |
| Seattle Biomedical Research Institute | Post-doc | 1985-1989 | Molecular Parasitology |

### A. Personal Statement

The goal of the proposed research is to perform a genome-wide survey to identify genes under translational control in the protozoan parasite, *Trypanosoma brucei*, by using the recently developed ribosome profiling technology. My role in this project will be to direct the next generation sequencing (NGS) and bioinformatics aspects of the project, and to partner with Dr. Marilyn Parsons, who will oversee the preparation of biological material. I have extensive experience (over 30 years) in parasite molecular biology, genomics and bioinformatics and, more recently, structural biology and drug development. After graduate work on *Plasmodium falciparum* (malaria) antigen identification, I undertook post-doctoral training in molecular biology; studying antigenic variation in African trypanosomes (*Trypanosoma brucei*) and *Anaplasma mariginale*. During the last post-doctoral period (at SBRI), I began to study *Leishmania* gene expression and developed expertise in DNA sequencing and bioinformatics. This led to my directing the *L. major* and *T. cruzi* genome sequencing projects at SBRI; an activity that continues until the present as a Co-PI on a trypanosomatid genomic database and annotation project. For the last 10 years, I have been at the forefront of applying genomic technologies such as microarray-based expression profiling and proteomics to increase our understanding of molecular mechanisms underlying trypanosomatid transcription and regulation of gene expression during differentiation. My laboratory has recently started making extensive use of NGS technology for genome re-sequencing, mRNA profiling (RNA-seq) and chromatin immunoprecipitation using sequencing (ChIP-seq) in several *Leishmania* species; once again being at the forefront of this technology for the trypanosomatid field. During this time, I also become involved in structural genomics, leading the Target Selection portion of the Structural Genomics of Pathogenic Protozoa (SGPP) project. I am currently PI and Director of the Seattle Structural Genomics Center for Infectious Disease (SSGCID), which is funded under a contract (HHSN272200700057C) from NIAID. The mission of SSGCID is to use X-ray crystallography and NMR spectroscopy to solve the structure of proteins targets in emerging and re-emerging infectious disease organisms in order to facilitate development of new therapeutics using structure-based drug design. I am also the Scientific Advisor for the Bioinformatics, Protein Production and DNA Sequencing Cores within SBRI's Global Health Biotechnology Center (GHBC).

### B. Positions and Honors

**Positions and Employment**

1990-1996 Associate Scientist, Seattle Biomedical Research Institute, Seattle, WA
1993-1997 Assistant Professor, Department of Pathobiology, University of Washington, Seattle, WA
1996-2000 Staff Scientist, Seattle Biomedical Research Institute, Seattle, WA.
1997-2004 Research Associate Professor, Department of Pathobiology, University of Washington, Seattle, WA
2000-2003 Associate Member, Seattle Biomedical Research Institute, Seattle, WA
2001-2004 Adjunct Research Associate Professor, Department of Medical Education and Biomedical Informatics, University of Washington, Seattle, WA

2004-present Full Member, Seattle Biomedical Research Institute, Seattle, WA
2004-2008 Research Professor, Department of Pathobiology, and Department of Medical Education and Biomedical Informatics, University of Washington, Seattle, WA
2007-2008 Adjunct Research Professor, Department of Global Health, University of Washington, Seattle, WA
2008-Present Affiliate Professor, Departments of Global Health & Medical Education and Biomedical Informatics, University of Washington, Seattle, WA

**Other experience and Professional Memberships**

1991-present Scientific Director, DNA Sequencing Core Facility, Seattle Biomedical Research Institute
2007-present Scientific Director, Protein Production Core, Seattle Biomedical Research Institute
2007-present Scientific Director, Bioinformatics Core, Seattle Biomedical Research Institute
2007-present Director, Seattle Structural Genomics Center for Infectious Disease (SSGCID)
1978-1981 Australian Biochemical Society
1995-present American Association for the Advancement of Science
1996-present The Society of Protozoologists
2004-present American Society for Microbiology
2007-present American Society of Tropical Medicine and Hygiene
1988-1992 USAID/AIBS, Malaria program review panel
1994-1995 NIH, Shared Instrumentation Grants Special Study Section
1999-2001 USDA, Sustaining Animal Health and Well-being (study section member)
2003 Leishmaniasis Review panel for Military Infectious Diseases Research Program of the US Army, Navy, and Air Force; Joint Medical Technology Workshop.
2001-present Editorial board, Kinetoplastid Biology and Disease

**Honors**

1986-present Invited speaker at 38 international meetings and session chair at 17 meetings
2008-2009 Member, Scientific Committee for 4th World Congress on Leishmaniasis

## B. Selected peer-reviewed publications (from 110 research papers and 10 reviews)

**Most relevant to the current application**


1. El-Sayed, N.M.A., **Myler, P.J.**, (42 other authors) and Hall, N. (2005) Comparative genomics of the trypanosomatids. **Science 306:**404-409.
2. Jensen, B.C., Sivam, D., Kifer, C.T., **Myler, P.J.**, and Parsons, M. (2009). Widespread variation in transcript abundance within and across developmental stages of *Trypanosoma brucei*. **BMC Genomic 10**:482. PMID: 19840382. PMCID: PMC2771046
3. [redacted]


**Additional recent publications (in chronological order)**


4. Rosenzweig, D., Smith, D., **Myler, P.J.**, Olafson, R.W., Zilberstein, D. (2008) Post-translational modification of cellular proteins during *Leishmania donovani* differentiation. **Proteomics 8:**1843-1850. PMID: 18398879
5. Thomas, S., Hitchcock, R.A., Anderson-Green, A., Sivam, D., Sturm, N.R., Campbell, D.A. and **Myler, P.J.** (2009) Histone acetylations mark origins of polycistronic transcription in *Leishmania major*. **BMC Genomics 10**:152. PMCID: PMC2679053
6. Padilla-Mejia, N.E., Florencio-Martinez, L.E., Figueroa-Angulo, E.E., Manning-Cela, R.G., Hernandez-Rivas, R., **Myler, P.J.** and Martinez-Calvillo, S. (2009) Gene organization and sequence analyses of transfer RNA genes in Trypanosomatid parasites. **BMC Genomics 10**:232. PMCID: PMC2695483
7. Buchko, G., Hewitt, S., Napuli, A., Van Voorhis, W., **Myler, P.J**. (2009) Backbone and side chain 1H, 13C, and 15N NMR assignments for the organic hydroperoxide resistance protein (Ohr) from *Burkholderia pseudomallei*. **Biomol NMR Assign. 3**:163-166. PMID: 19888681. PMCID: PMC2774895
8. **Myler, P.J.**, Stacy, R., Stewart, L., Staker, B., Van Voorhis, W.C., Varani, G., and Buchko, G.W. (2009) The Seattle Structural Genomics Center for Infectious Disease (SSGCID). **Infectious Disorders - Drug Targets 9**:493-506. PMID: 19594426. PMCID: PMC2857597

9. Van Voorhis, W.C., Hol, W.G.J., **Myler, P.J.** and Stewart, L.J. (2009) The role of Medical Structural Genomics in discovering new drugs for infectious diseases. **PLoS. Comput. Biol. 5**:e1000530. PMID: 19855826. PMCID: PMC2756625
10. Aslett, M., (25 other authors), **Myler, P.J.**, (19 other authors). (2010) TriTrypDB: a functional genomic resource for the Trypanosomatidae. **Nucleic Acids Res. 38**:D457-D462. PMID: 19843604. PMCID: PMC2808979

## C. Research Support

**Ongoing Research Support**

R01 AI053667 Myler (PI) 1/1/03 – 4/30/11
NIH/NIAID
**Transcription of Protein-coding Genes in *Leishmania***
The major goals of this project are to characterize the components of the RNA polymerase II transcription complex in *Leishmania major* and to elucidate the molecular mechanisms involved in pol II-mediated transcription of protein-coding genes.

HHSN272200700057C Myler (PI) 9/28/07 – 9/27/12
NIH/NIAID
**Center for Structural Genomics of Infectious Diseases**
The primary goal of this project is to determine the structure of 75-100 protein targets from NIAID Category AC and emerging/re-emerging infectious disease organisms each year for a period of five years. This will be accomplished by employing a high-throughput gene-to-structure pipeline involving a multi-pronged serial escalation approach to protein expression followed by structure solution using X-ray crystallography and NMR spectroscopy.

[redacted]

**Completed Research Support** (since 2007)

[redacted]

## 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 |
|---|---|
| Marilyn Parsons, Ph.D. | Member and Director of Scientific Operations |
| 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 |
|---|---|---|---|
| University of Kansas, Lawrence, Kansas | B.A. | 1974 | Biology |
| Stanford University, Stanford, California | Ph.D. | 1979 | Genetics |

### A. Personal statement

The goals of this project are to adapt a new ribosome profiling technology to *Trypanosoma brucei* and to exploit this approach to assess the role of translational control in stage-specific gene regulation. A corollary is to provide information related to relative translation rates as a surrogate for protein abundance. My group has considerable expertise in the cell and molecular biology of *T. brucei*, initiating with my own training in molecular Parasitology in Dr. Nina Agabian's lab as a postdoc, where I studied antigenic variation and trans-splicing. My first paper as an independent scientist dealt demonstrated translational control of phosphoglycerate kinase in trypanosomes (publication 1 below), and subsequently we demonstrated translational control of a protein kinase NrkA (publication 2). Additionally, we have shown the polysome profiles change during parasite development, indicating stage-regulation of ribosome loading (publication 3). Since establishing my lab, I have been continuously funded by NIH to work on trypanosomes, and more recently *Toxoplasma*. I have demonstrated my skills in project management over the years and have assembled an excellent staff. I work in an outstanding collaborative environment at Seattle BioMed, where two other labs (Stuart and Myler groups) conduct work on trypanosomatids, allowing us to make use of the latest cell, molecular, and bioinformatic approaches. For the proposed project, we are collaborating with Dr. Peter Myler who has considerable experience in bioinformatics and sequencing, including recent forays into next generation sequencing. Dr. Myler and I collaborated on a recently published project that examined gene regulation at the mRNA abundance level during *T. brucei* development using microarray technology (publication 12) and we are currently collaborating on establishing a novel gene regulatory system in *Leishmania*. Our contributions to the proposed studies will focus on parasite biology.

### B. Positions and Honors

**Positions**

| | |
|---|---|
| 1974-1979 | Graduate research in human genetics with Dr. L.L. Cavalli-Sforza, Stanford University. |
| 1979-1981 | Bank of America-Giannini Medical Research Fellow in Immunogenetics with Drs. Leonard and Leonore Herzenberg, Stanford University. |
| 1981-1985 | Senior fellow in Biochemistry with Dr. Nina Agabian, University of Washington. |
| 1985-2000 | Senior Scientist, Seattle Biomedical Research Institute |
| 1986-1991 | Research Assistant Professor, Department of Pathobiology, University of Washington |
| 1988-2008 | Associate Director, Seattle Biomedical Research Institute |
| 1991-1994 | Research Associate Professor, Department of Pathobiology, University of Washington |
| 1994-1996 | Associate Professor, Department of Pathobiology, University of Washington |
| 1996-2008 | Professor, Department of Pathobiology, University of Washington |
| 2000-Present | Full Member, Seattle Biomedical Research Institute |
| 2008-Present | Fogarty Global Infectious Diseases Training Grant, Co-Director |
| 2008-Present | Director of Scientific Operations, Seattle Biomedical Research Institute |
| 2008-Present | Affiliate Professor, Department of Global Health, University of Washington |

**Honors and Professional Activities**

| | |
|---|---|
| 1978-1980 | National Science Foundation Predoctoral Fellowship |
| 1979-1981 | Bank of America-Giannini Foundation Medical Research Fellowship |
| 1979 | Katherine D. McKormick Fellowship |

| | |
|---|---|
| 1995-1998 | NIH Tropical Medicine and Parasitology Study Section Member |
| 2000 | NIH Fogarty International Research Collaboration Award Study Section, ad hoc |
| 2002-2004 | NIH Fogarty International GRIP Special Study Section |
| 2001-Present | Faculty of 1000 |
| 2002-2008 | Military Infectious Disease Research Program, Malaria Drug Development Review Panel |
| 2002 | NIAID Loan Repayment Special Study Section |
| 2005 | NIH AIDS Opportunist and Cancer Study Section, ad hoc |
| 2007-9 | ASM Division AA Parasitic, symbiotic, and free-living protists. Chair-elect; Chair, Colloquium Committee. |
| 2009-Present | Joint Chief Editor, Molecular and Biochemical Parasitology |

Editorial Boards: Eukaryotic Cell; PLoS Pathogens

**c. Selected peer-reviewed publications (out of 105 total)**


1. **Parsons, M.**, and Hill, T. (1989) Elevated phosphoglycerate kinase mRNA but not protein in monomorphic *Trypanosoma brucei*: implications for stage-regulation and post-transcriptional control. Mol. Biochem. Parasitol. 33:215-228.
2. Gale, M., Jr., Carter, V., and **Parsons, M.** (1994) Translational control mediates the developmental regulation of the *Trypanosoma brucei* Nrk Protein Kinase. J. Biol. Chem. 269:31659-31665.
3. Brecht, M., and **Parsons, M.** (1998) Changes in polysome profiles accompany trypanosome development. Mol. Biochem. Parasitol. 97: 189-198.
4. Jensen, B., Wang, Q., Kifer, C., and **Parsons, M.** (2003) The NOG1 GTP-binding protein is required for biogenesis of the 60S ribosomal subunit. J. Biol. Chem.378: 32204-32211.
5. DeRocher, A., Gilbert, B., Feagin, J.E., and **Parsons, M.** (2005). Dissection of brefeldin A sensitive and insensitive steps in apicoplast protein targeting. J. Cell Sci., 118: 565-574.
6. Jensen, B.C., Brekken, D.L., Randall, A.C., Kifer, C.T., and **Parsons, M**. (2005) Species specificity in ribosome biogenesis: a non-conserved phosphoprotein is required for 60S biogenesis in *Trypanosoma brucei.* Eukaryotic Cell, 4:30-35. PMCID: PMC544161.
7. El-Sayed,N.M.A., Myler,P.J., Bartholomeu,D., Nilsson,D., Aggarwal,G., Tran,A.-N., Ghedin,E., Worthey,E.A., Delcher,A., Blandin,G., Westenberger,S., Haas,B., Caler,E., Cerqueira,G., Arner,E., Aslund,L., Bontempi,E., Branche,C., Bringaud,F., Campbell,D., Carrington,M., Crabtree,J.S., Darban,H., Edwards,K., Englund,P., Feldblyum,T., Ferella,M., Frasch,C., Kindlund,E., Klingbeil,M.M., Kluge,S., Koo,H.L., Lacerda,D., McCulloch,R., McKenna,A., Mizuno,Y., Mottram,J., Ochaya,S., Pai,G., **Parsons,M**., Pettersson,U., Pop,M., Luis Ramirez,J., Salzberg,S., Tammi,M., Tarleton,R.L., Teixeira,S.M., Van Aken,S., Wortman,J., Stuart,K.D., Andersson,B., Anapuma,A., Attipoe,P., Burton,P., Cadag,E., Franco da Silva,J., de Jong,P., Fazelinia,G., Gull,K., Horn,D., Hou,L., Huang,Y., Levin,M.J., Lorenzi,H., Louie,T., Machado,C.R., Nelson,S., Osoegawa,K., Pentony,M., Rinta,J., Robertson,L., Sanchez,D.O., Seyler,A., Sharma,R., Shetty,J., Simpson,A.J., Sisk,E., Vogt,C., Ward,P., Wickstead,B., White,O., Fraser,C.M., Stuart,K.D.; and Andersson,B. (2005). The genome sequence of *Trypanosoma cruzi*, etiological agent of Chagas' disease. Science 309: 409-415.
8. **Parsons, M.,** Worthey, E.A., Ward, P.N., and Mottram, J.C. (2005) Comparative analysis of the kinomes of three pathogenic trypanosomatids: *Leishmania major, Trypanosoma brucei*, and *Trypanosoma cruzi*. BMC Genomics 6: 127-. PMCID: PMC1266030
9. Naula, C., **Parsons, M**., and Mottram, J.C. (2005) Protein kinases as drug targets in trypanosomes and *Leishmania*. Biochim. Biophys. Acta, 1754: 151-159. PMCID: PMC1452262

10. Jensen, B.C.,Kifer, C.T., Brekken, D.L., Randall, A.C., Wang, Q., Drees, B.L., and **Parsons, M.** (2007) Characterization of Protein Kinase CK2 from *Trypanosoma brucei*. Mol. Biochem. Parasitol. 151: 28-40. PMCID: PMC1790856.

11. Haanstra, J., van Tuijl, A., Kessler, P.S., Reijnders, W., Michels, P., Westerhoff, H.V., **Parsons, M.**, Bakker, B. (2008) Compartmentation prevents a lethal turbo-explosion of glycolysis in trypanosomes. Proc. Natl. Acad. Sci. USA, 105: 17718-23. PMCID: PMC2584722

12. Jensen, B.C., Sivam, D., Kifer, C.T., Myler, P.J., and **Parsons, M.** (2009). Widespread variation in transcript abundance within and across developmental stages of *Trypanosoma brucei*. BMC Genomics 2009, 10:482. PMCID: PMC2771046

13. Ojo KK, Larson ET, Keyloun KR, Castaneda LJ, Derocher AE, Inampudi KK, Kim JE, Arakaki TL, Murphy RC, Zhang L, Napuli AJ, Maly DJ, Verlinde CL, Buckner FS, Parsons M, Hol WG, Merritt EA, Van Voorhis WC. Toxoplasma gondii calcium-dependent protein kinase 1 is a target for selective kinase inhibitors. Nat Struct Mol Biol. 2010 May;17(5):602-7. NIHMSID # 203016.

14. Worthen, C., Jensen, B.C., and **Parsons, M. (2010)** Diverse effects on mitochondrial and nuclear functions elicited by drugs and genetic knockdowns in bloodstream stage *Trypanosoma brucei*, PLoS NTDs, 4:e678. PMCID: PMC2864271.

15. [redacted]

## D. Research Support

R01 AI050506 (Parsons, P.I.) 4/1/08-3/31/13
NIH/NIAID
**The Plastid of *Toxoplasma gondii***
This project proposes to examine a plant-like organelle called the apicoplast in the parasite *Toxoplasma gondii*, an agent which causes encephalitis in immunocompromised individuals and birth defects when acquired during pregnancy. As humans lack an apicoplast, it provides a potential target for new interventions against the parasite.

R01 AI031077 (Parsons, P.I.) 2/1/92-1/31/11
NIH/NIAID
**Protein Phosphorylation in Trypanosomes**
The major goals of this project are to study the role of protein protein kinases and phosphatases in African trypanosomes. The project focuses on a bioinformatic analysis of protein phosphatases and functional studies of membrane and dual specificity kinases.

R01 AI069057-02 (Parsons, P.I.) 5/1/07-4/30/12
NIH/NIAID
**Mitochondrial Function in Bloodstream *Trypanosoma brucei***
*Trypanosoma brucei* is a protozoan pathogen that causes diseases in man and livestock. Most drugs are increasingly ineffective due to toxicity and development of resistance. The goal of this project to understand mitochondrial function in slender bloodstream form *T. brucei*, its role in the action of existing drugs, and its potential as a target for new drugs.

U01 AI075641 (Stuart, P.I) 9/1/07-8/31/12
NIH/NIAD
**Trypanosomatid Drug Development Consortium**
The project is designed to generate a robust pipeline of anti-trypanosomatid drugs with product profiles suitable for clinical trials and deployment. It will be conducted by a Consortium which includes SBRI/University

of Washington, UCSF, University of North Carolina at Chapel Hill, and the Eskitis Institute of Griffiths University. The project will use a milestone based, decision matrix supported, product development approach to: 1) Identify and prioritize trypanosomatid candidate drug targets 2) Develop high throughput screens for prioritized targets 3) Screen selected libraries 4) Perform hit-to-lead chemistry 5) Perform initial efficacy and toxicology studies and 6) Generate a centralized project and information management system. The outcome will be sets of targets and chemical entities distributed along the pipeline from target discovery to preclinical validation thus providing a rich ongoing resource of drug candidates for clinical studies. Role: Scientific Lead, SBRI.

D43 TW000924 (Stuart, P.I.) 8/25/06-3/31/11
NIH/Fogarty International Center
**Research Training on Intracellular Pathogens**
This project is a training grant which fosters research collaborations between two Seattle institutions (Seattle Biomedical Research Institute and the University of Washington) and two New Delhi institutions (Jawaharlal Nehru University and the International Center for Genetic Engineering and Biotechnology, ICGEB). Students and postdoctoral fellows work on collaborative projects focusing on intracellular pathogens for periods of 6 months to one year. In country workshops are also conducted. Role: US Co-Director.

# PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix:
* First Name: Peter
Middle Name: John
* Last Name: Myler
Suffix: PhD

## 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:
* First Name: Jennifer
Middle Name:
* Last Name: Dodson
Suffix:

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

* Title: Manager, Grants and Contracts

* Street1: 307 Westlake Ave N, Suite 500
Street2:
* City: Seattle
County/Parish: King
* State: WA: Washington
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 98109-5219

# 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:GRANT10631552 Funding Opportunity Number:PA-10-069 Received Date:2010-06-15T15:58:06-04:00

# PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

## Budget Period: 1

Start Date: 04/01/2011 End Date: 03/31/2012

### A. Direct Costs

| | * Funds Requested ($) |
|---|---|
| * Direct Cost less Consortium F&A | [redacted] |
| Consortium F&A | |
| * Total Direct Costs | [redacted] |

### B. Indirect Costs

| | Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|---|
| 1. | Modified Total Direct Cost Base | [redacted] | [redacted] | [redacted] |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Ernest L. Willard, 415-437-7820

Indirect Cost Rate Agreement Date: 07/20/2009

Total Indirect Costs: [redacted]

### C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

## Budget Period: 2

Start Date: 04/01/2012 End Date: 03/31/2013

### A. Direct Costs

| | * Funds Requested ($) |
|---|---|
| * Direct Cost less Consortium F&A | [redacted] |
| Consortium F&A | |
| * Total Direct Costs | [redacted] |

### B. Indirect Costs

| | Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|---|
| 1. | Modified Total Direct Cost Base | [redacted] | [redacted] | [redacted] |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Ernest L. Willard, 415-437-7820

Indirect Cost Rate Agreement Date: 07/20/2009

Total Indirect Costs: [redacted]

### C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

# PHS 398 Modular Budget, Periods 3 and 4

**Budget Period: 3**

Start Date: End Date:

**A. Direct Costs**

| | * Funds Requested ($) |
|---|---|
| * Direct Cost less Consortium F&A | |
| Consortium F&A | |
| * Total Direct Costs | |

**B. Indirect Costs**

| | Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|---|
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

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

Indirect Cost Rate Agreement Date

Total Indirect Costs

**C. Total Direct and Indirect Costs (A + B)** Funds Requested ($)

**Budget Period: 4**

Start Date: End Date:

**A. Direct Costs**

| | * Funds Requested ($) |
|---|---|
| * Direct Cost less Consortium F&A | |
| Consortium F&A | |
| * Total Direct Costs | |

**B. Indirect Costs**

| | Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|---|
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

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

Indirect Cost Rate Agreement Date

Total Indirect Costs

**C. Total Direct and Indirect Costs (A + B)** Funds Requested ($)

# PHS 398 Modular Budget, Periods 5 and Cumulative

**Budget Period: 5**

Start Date: End Date:

**A. Direct Costs**

| | * Funds Requested ($) |
|---|---|
| * Direct Cost less Consortium F&A | |
| Consortium F&A | |
| * Total Direct Costs | |

**B. Indirect Costs**

| | Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|---|
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

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

Indirect Cost Rate Agreement Date

Total Indirect Costs

**C. Total Direct and Indirect Costs (A + B)**

Funds Requested ($)

**Cumulative Budget Information**

**1. Total Costs, Entire Project Period**

| | |
|---|---|
| *Section A, Total Direct Cost less Consortium F&A for Entire Project Period | $ [redacted] |
| Section A, Total Consortium F&A for Entire Project Period | $ |
| *Section A, Total Direct Costs for Entire Project Period | $ [redacted] |
| *Section B, Total Indirect Costs for Entire Project Period | $ [redacted] |
| *Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period | $ [redacted] |

**2. Budget Justifications**

| | | | | |
|---|---|---|---|---|
| Personnel Justification | Personnel_justification1003112 | Add Attachment | Delete Attachment | View Attachment |
| Consortium Justification | | Add Attachment | Delete Attachment | View Attachment |
| Additional Narrative Justification | Additional_Narrative_Justifica | Add Attachment | Delete Attachment | View Attachment |

## Personnel Justification

*Peter Myler, Ph.D., Principal Investigator, 1.2 calendar months/10% effort*. Dr. Myler will provide expertise in bioinformatics and genomics. He will act as the project's Principal Investigator along with Dr. Parsons. He will work with Dr. Parsons to supervise the project, including scientific and management oversight and manuscript preparation. Dr. Myler will be the contact PI for grant reporting.

*Marilyn Parsons, Ph.D., Principal Investigator, 0.84 calendar months/7% effort*. Dr. Parsons will provide expertise in *T. brucei*, including parasite development. Together with Dr. Myler, she will act as Principal Investigator and will supervise the project, including scientific and management oversight, experimental design, troubleshooting, and manuscript preparation.

*Bryan Jensen, Ph.D., Staff Scientist, 1.2 calendar months/10% effort*. Dr. Jensen has extensive experience in research on African trypanosomes, including animal infections and polysome analysis. He will assist in experimental design and daily supervision of the other staff on the project. Dr. Jensen will also be responsible for generation of parasites from *in vitro* culture and from animal infections.

*Andrew Haydock, Research Technician II, 6 calendar months/50% effort.* Mr. Haydock will isolate polysomes and RNA, prepare the RNA-seq libraries and interface with the Bioinformatics Core personnel for analysis of the NGS reads. He has extensive experience in NGS library preparation and analysis, having generated all the *Leishmania* RNA-seq data for the Myler Lab.

Benefits are calculated at a rate of 25.4% of base salary in accord with Seattle BioMed's nonprofit rate agreement dated 07/20/09.

The approaches provide a strong partnership that will allow application of this novel technology and further understand the significance of finding with respect to translational control in *T. brucei*.

**Bioinformatics**

Dr. Myler serves as the Scientific Advisor for the Bioinformatics Core at Seattle BioMed. It employs several experienced programmers who can develop new scripts and pipelines, which may be required by some project studies. By using the Core on a fee for-service basis, the project will gain the relevant expertise from a number of individuals, depending on project needs, in a cost-effective manner. The total funds for the Core are approximately equivalent to 3.5 calendar months per year. An RNA-seq pipeline already developed by Core staff will be adapted as needed to manage the results of the sequencing of the libraries. Dr. Myler and the Bioinformatics Core are also key participants in the development of TriTrypDB, through which data from the project will be made available.

**Additional Narrative Justification**

We are requesting that in the R21 budget, six modules ($[redacted] be budgeted to Year One and five modules ($125,000) be budgeted to Year Two. In year one, expenses will be slightly higher due to developing the Bioinformatics pipeline and the purchase of a cell grinder. Our total costs for both years will be $275,000.

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 | 2_Specific_Aims1003112211.p | Add Attachment | Delete Attachment | View Attachment |
| 3. *Research Strategy | 3_Research_Strategy10031122 | 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 | 10_Vertebrate_animals100311 | Add Attachment | Delete Attachment | View Attachment |
| 11. Select Agent Research | | Add Attachment | Delete Attachment | View Attachment |
| 12. Multiple PD/PI Leadership Plan | 12_Multiple_PI_Plan10031121 | Add Attachment | Delete Attachment | View Attachment |
| 13. Consortium/Contractual Arrangements | | Add Attachment | Delete Attachment | View Attachment |
| 14. Letters of Support | 14_LettersofSupport100311221 | Add Attachment | Delete Attachment | View Attachment |
| 15. Resource Sharing Plan(s) | 15_resource_sharing10031121 | Add Attachment | Delete Attachment | View Attachment |

16. Appendix Add Attachments | Remove Attachments | View Attachments

## 2. Specific Aims

Gene expression in trypanosomatids (such as *Trypanosoma brucei* and the various *Leishmania* species) is distinct from other well-studied eukaryotes because the protein-coding genes are transcribed polycistronically. However, co-transcribed mRNAs encode proteins that display dramatic variation in abundance both within and across developmental stages, indicating that post-transcriptional controls provide the major means of regulating expression of individual genes. Our previous microarray study has shown significant differences in mRNA abundance within and across *T. brucei* bloodstream and insect stages (likely reflecting differences in mRNA stability), while other studies have identified considerable changes in the proteome. A recent global analysis of mRNA levels and protein abundances (from the same biological samples) at several time-points during promastigote-to-amastigote differentiation of *L. donovani* (conducted by the Myler lab) showed that the correlation between these is rather low. However, both microarrays and proteomic analysis are limited by a lack resolution in quantitation of lower abundance molecules, leaving the true correlation between mRNA and protein levels open to question. Furthermore, other data suggests that translational and/or post-translational controls also play significant roles. For example, in-depth analysis (by the Parsons lab) of two *T. brucei* genes demonstrated translational control as a key mechanism. We therefore hypothesize that translational controls function both to tune the levels of protein within stages and to change the levels across stages. This project seeks to address this hypothesis by quantitatively assessing the rate at which cellular mRNAs are being actively translated at any particular time. This will be accomplished by adapting and 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 (NGS). To establish the system and test our hypothesis for *T. brucei*, we propose the following Specific Aims.

**Aim 1. Establish the ribosome protection assay in *T. brucei* strain 927 cultured procyclic forms.** Optimization of footprinting, library construction and informatics will be done using cultured log-phase procyclic forms, which are readily available under standardized conditions. Cell lysates will be treated with RNase I and ribosome-protected RNA fragments will be isolated and used to generate libraries for sequencing *via* Illumina NGS technology. The resulting data will be entered into our RNA-seq pipeline and aligned with the *T. brucei* genome to identify the number and location of ribosomes that are bound to gene-specific mRNA. This data will indicate the level of gene-specific translation for every gene detected, as well as identifying the specific sequences on each mRNA that are translated. Comparison with the profile of total cellular mRNA will establish the translational efficiency of transcripts corresponding to specific genes.

**Aim 2. Identify genes that are regulated at the level of translation during *T. brucei* development.** We will carry out similar studies on rapidly-dividing, mammalian-infective slender bloodstream forms and non-dividing stumpy bloodstream forms from animals. Comparison of the ribosome profile of mRNAs at these stages and that of procyclic forms (from Aim 1) will identify genes that are regulated at the level of translation.

The proposed work promises to provide an important new tool for studying trypanosomatid gene expression, yielding clues to the mechanism of translational control in trypanosomatids, and new information on the extent of translation of individual gene products. 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 open-reading frames, thus significantly enhancing the ongoing genome annotation. We also anticipate that this technology will be very useful to those researchers wishing to determine which trypanosomatid proteins are likely to be present in infective stages, and thus might serve as drug and vaccine targets.

## 3. RESEARCH STRATEGY

# a. Significance

*Trypanosoma brucei* is the causative agent of African trypanosomiasis ("sleeping sickness"). The number of human deaths caused annually by this species is estimated at 50,000, although over 6 million people in sub-Saharan Africa are at risk. The impact of sleeping sickness is high, due to the cost of treatment combined with high morbidity and mortality [1]. Related pathogens include *Trypanosoma cruzi* (the causative agent of Chagas' disease) and numerous *Leishmania* species, which cause a diverse spectrum of visceral, mucocutaneous, and cutaneous disease. Each of these parasites undergoes a complex developmental cycle, alternating between mammalian and insect hosts, as well as proliferating and non-proliferating stages. The stages of *T. brucei* available in the laboratory are slender (dividing) bloodstream forms, stumpy (non-dividing) bloodstream forms, and cultured procyclic forms (which correspond to the stage found in the tsetse fly midgut). These stages differ significantly in morphology, surface antigen expression, and metabolic functions [2]. In naturally occurring infections, slender bloodstream forms generally exist at low parasitemias, and through a relatively poorly-defined quorum sensing mechanism [3], differentiate to non-dividing stumpy forms, which are thought to be pre-adapted metabolically to survive upon ingestion by the tsetse fly. Most laboratory strains have lost the ability to differentiate into stumpy forms and hence are called "monomorphic" strains, in contrast to their "pleiomorphic" ancestors.

Exactly how trypanosomatid gene expression gives rise to the different stage-specific phenotypes is not well understood. In contrast to mammals and fungi, the relative contribution of gene-specific transcriptional control appears low in trypanosomatids, since the protein-coding genes are organized in large polycistronic clusters [4-8] , and mRNAs derived from the same transcriptional unit vary widely in abundance within and between developmental stages [9-11]. Differences in post-transcriptional processing and stability play major roles in regulating mRNA abundances in *T. brucei* and *Leishmania* [12-16], but comparison of microarray and proteomic analyses showed that there is a poor correlation between mRNA and protein abundance during *Leishmania* development [17]. Indeed, our own study [18] using the same biological samples from several time points during promastigote-to-amastigote differentiation indicated that almost a third of the 902 genes analyzed showed poor (or negative) correlation between changes in mRNA and protein levels (**Fig. 1**). Several studies, including our own, have provided evidence for translational control of stage-regulation in *Leishmania* [19,20] and *T. brucei* [13,21,22]. We have also shown that stumpy form *T. brucei* have a paucity of polysomes [23], indicating developmental changes in translational capacity. These findings suggest that translational controls may figure prominently in modulating protein abundance in trypanosomatids. Similar layered controls have been observed in other organisms, such as *Saccharomyces*, in which the correlation of mRNA to protein abundance can be less than 0.2 [24].

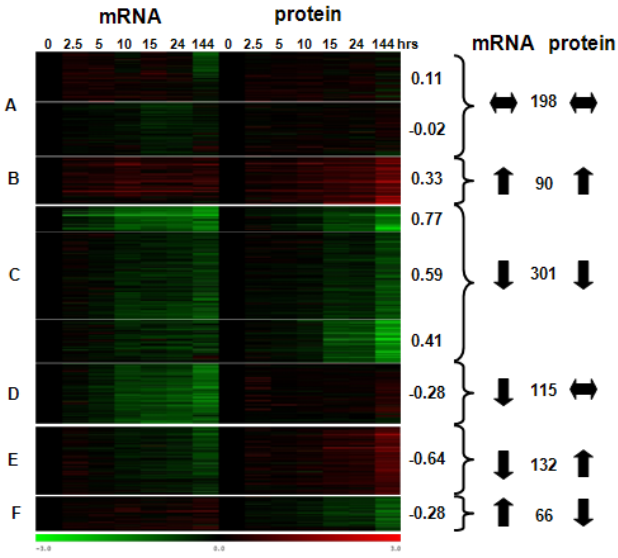


**Figure 1.** Cluster analysis of mRNA and protein co-expression during *Leishmania* differentiation
K-median clustering of mRNA and protein abundance changes was performed using TMeV software to produce nine clusters with six different patterns (letters on the left). The average correlation co-efficient between changes in mRNA and protein abundance is shown to the right of each cluster. The number of genes in each cluster pattern is shown further to the right, between arrows indicating the general change in mRNA and protein abundance.

While recent proteomic analyses have begun to elucidate some developmentally regulated proteins in trypanosomatids [17,25-27], this approach remains

challenging for low abundance proteins, precluding global analysis. However, recent developments allow the global measurement of the translational status of mRNAs by profiling, at the codon level, their occupation with ribosomes [24]. Ribosomes assembled onto mRNA protect about 28 nt from exogenous nuclease. Thus, if polysomal RNA is treated with nuclease, the segments of mRNAs actively undergoing translation are preserved, while the rest of the mRNA is degraded. By sequencing the protected fragments, one can obtain a measure of the relative ribosome loading of the transcript, and also the location of the ribosomes along the mRNA. The power of next generation sequencing (NGS) allows this technology to penetrate to low abundance mRNAs, opening up possibilities that are not available through other approaches, such as microarray analysis of polysomal fractions. In addition, since ribosome profiling has resolution at the codon level, it enables much more precise interrogation of exactly which open reading frames (ORFs) are being actively translated at any given, rather than just revealing which mRNAs are associated with ribosomes as in the case of microarray-based analyses. Thus, this exciting technological advance will allow us to assess for the first time the relative translation of *T. brucei* mRNAs as compared to their relative abundance; taking us one step closer to the major mediator of function, the protein itself.

Here we propose to adapt the ribosome profiling technology to *T. brucei*, and then to assess the ribosome occupancy of mRNAs across parasite development. The results will be compared to the relative mRNA levels in those same samples. The data will identify those transcripts that are preferentially translated, as well as those whose translation differs among stages, thereby indicating the extent of translational control in parasite development. It will also clarify whether the many "novel genes" identified through RNA-seq experiments [11] are actively translated, thus adding to the repertoire of experimentally validated protein-coding genes. This project will also be relevant to drug target discovery, since it will identify those transcripts that likely result in protein products in the mammalian infective stages, as opposed to those which remain silent within the mRNA.

## b. Innovation

The control of gene expression in trypanosomatid parasites is relatively poorly understood. While mRNAs within the same transcription unit vary in abundance within and across developmental stages, the impact of translational control on protein abundance remains unexplored except for a few cases. This application proposes to adapt a new technology that melds advances in NGS with ribosome protection of translating mRNAs to reveal the global extent of translational control in the organism chosen as a model trypanosomatid, *T. brucei*. The studies will provide important new information at several levels: it will identify transcripts that are under stage-regulated translational control and also those transcripts that are more (or less) efficiently translated in all stages. Additionally, it will assess whether numerous small putative coding regions (CDSs) identified in RNA-seq experiments are translated, and whether some transcripts bear upstream ORFs that may function in translational control. Finally, it will determine whether *T. brucei* employs alternative initiation codons, such as were recently revealed to be much more prominent than suspected in *S. cerevisiae* [24]. The expertise of Dr. Parsons in *T. brucei* and translation and Dr. Myler in bioinformatic/genomic approaches provide a strong partnership that will allow application of this novel technology and substantially increase our understanding of translational control in *T. brucei*. These studies are anticipated to be a prelude to future studies examining the roles of specific sequences and proteins mediating translational regulation in *T. brucei*. They also will provide a strong foundation for exploring translational control in other trypanosomatid parasites, specifically *Leishmania donovani*.

## c. Proposed research approach

The overall goal of this project is to adapt a technology for measuring ribosome occupation of mRNAs to *T. brucei* and to use it to examine the relative translation of mRNAs within and across the life cycle of the parasite, providing insights into gene regulation and coding potential of the parasite genome. *T. brucei* was

selected for study because different developmental stages can be obtained in the laboratory in quantities that will allow development of the procedures and their initial application.

**Aim 1. Establish the ribosome protection assay in *T. brucei* strain procyclic forms.**

Our project will begin by adapting the ribosome profiling technology to *T. brucei*. We will employ strain 927, since it can be cultured as both procyclic (insect) and slender (mammalian) bloodstream forms *in vitro*, and can be grown as slender and stumpy bloodstream forms in rats. In addition, this strain has the most complete genome sequence [4]. In order to establish the optimal conditions for ribosome profiling, the goal of Aim 1, we will use cultured procyclic forms in log phase, because these are easy (and less expensive) to obtain under standardized conditions in culture. One liter of cultured procyclic forms will provide sufficient material for multiple analyses. Our procedure is based on a 50-page protocol (summarized in **Fig. 2**) provided by Dr. Nicholas Ingolia, corresponding author of the recent *Science* paper describing this technology [24]. Dr. Ingolia has agreed to advise us on this project (see letter of support). Although the protocol will be an excellent starting point for our experiments, we anticipate that certain steps will require further optimization, as addressed below.

**Preparation of RNA samples:** Procyclic form parasites will be treated with cycloheximide in culture medium to arrest translation while maintaining ribosome positioning. To prevent changes in ribosome loading, rapid processing is essential. Parasites will collected by filtration and scraped into a previously optimized polysome buffer [23], flash-frozen and ground under liquid nitrogen. A small fraction of the sample will be used for standard mRNA preparation using magnetic oligo-dT Dynabeads (Invitrogen) and subjected to alkaline cleavage to sizes of approximately 28 nt. Of the remainder, half will be left intact and half will be cleaved with RNase I, which will degrade RNA that is not protected by ribosomes (RNase I has little sequence specificity making it ideal for this purpose). The ribosomal fraction of the latter two samples (monosomes and polysomes in the undigested sample, and monosomes only in the RNase-digested sample) will be prepared by density gradient fractionation using procedures in place in the Parsons laboratory and RNA prepared [23,28]. Intact polyA+ RNA will be collected from the undigested sample and cleaved by alkaline lysis, while ribosome-protected RNA fragments will be prepared from the RNase-digested sample. This procedure will thus yield three preparations: randomly-cleaved total mRNA, randomly-cleaved mRNA associated with ribosomes, and 28-nt regions of mRNA protected by ribosomes. Each of these will be used to generate RNA-seq libraries for subsequent NGS and analysis as described below.

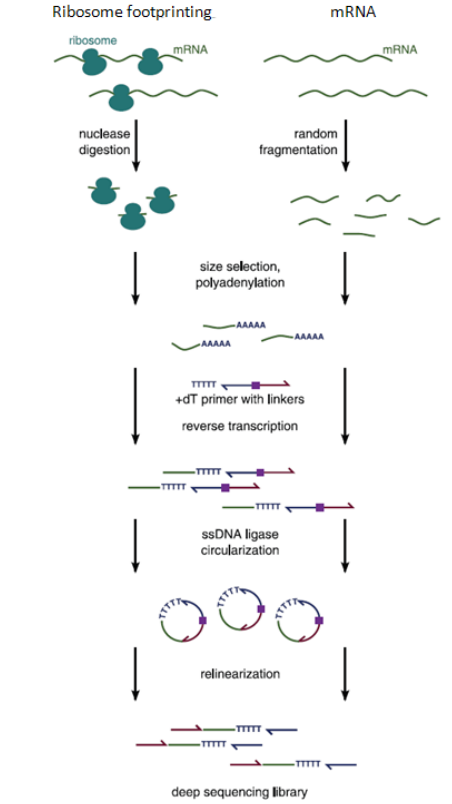


**Figure 2.** Ribosome profiling protocol
This schematic summarizing the protocol is taken from Ingolia *et al*. Note that libraries are constructed from the RNA fragments protected from nuclease digestion by ribosomes (left), and alkali-digested total or ribosome-associated mRNA (right). The gradient centrifugation step to isolate the ribosomal fraction is not shown.

*Optimization of nuclease digestion:* A specific challenge is optimizing nuclease digestion such that unprotected RNA is completely digested; while maintaining the yield of ribosome-protected RNA. To choose the best conditions for cleavage of polysomal RNA to "footprint"-sized molecules, the size of the resulting RNA fragments will be assessed by RNase protection assay, using an *in vitro*-transcribed, [$^{32}$P]-labeled fragment of *T. brucei* β-tubulin as the target.

**RNA-seq library preparation:** The 28-nt fraction of the three RNA preparations will be gel-purified and used to create libraries by the single-stranded (ss) DNA ligase method, which was demonstrated to avoid the sequence bias characteristic of RNA ligases [24]. In brief, the RNA fragments are polyadenylated and DNA synthesis primed with an oligo-$dT_{20}$ oligonucleotide ("dSpacer") containing bi-directional sequencing primers separated by an abasic furan (see **Fig. 2**). The cDNA is then circularized with ssDNA ligase (CircLigase, Epicentre), linearized within the dSpacer using APE1 endonuclease (New England Biolabs), and gel purified prior to amplification. To avoid bias, sequencing reactions will be performed on amplified samples which have maximized abundance of full-length product, but which still retain unincorporated primers.

While this protocol will ensure the most unbiased representation of mRNA abundance, we will compare it to a simpler method that we have developed for preparation of NGS libraries from intact total and ribosome-associated mRNA samples, which exploits the fact that all trypanosomatid mRNA bear a common 5' 39-nt spliced leader (SL) sequence. First strand cDNA synthesis using random hexamer-containing primers and 2nd strand synthesis using an SL-containing primer, followed by amplification and sequencing using an SL primer has allowed us to obtain more than 20 million sequence tags in a single NGS run from less than 1 μg of total RNA without mRNA enrichment. If this methodology provides us with similar relative mRNA abundances to that observed for using the ssDNA ligase method, we will employ it in Aim 2, since it uses less sample, as well as being less costly and labor-intensive.

*Contamination with non-coding RNAs:* The ribosome footprint libraries will almost certainly be contaminated with rRNA (it represented approximately 80% of the sequences obtained from the yeast experiments) and small RNAs. *T. brucei* possesses a robust RNAi system that generates small interfering RNAs, which are predominantly double-stranded fragments of 24-26 nt corresponding to the SLACs and INGI retroposon-like elements [29]. While ~10% of siRNAs are associated with polysomes [30], they are obviously not translated and are only a minor fraction of RNA that is associated with ribosomes. Nevertheless, we will examine the ribosome footprint library sequences for an over-representation of SLACs sequences derived from ORF1 (from whence most siRNAs are derived) [31] as compared to ORF2, to determine whether siRNAs represent a major contaminant in our RNA-seq library. While these contaminating sequences can readily be removed computationally; we will remove rRNA fragments by capture with complementary biotinylated oligonucleotides or by degradation of abundant sequences by duplex-specific nuclease (Illumina), if necessary. In addition, siRNAs can be removed by differential salt-extraction [30]. The SL-seq approach minimizes contamination, but cannot be used for the ribosome footprint samples.

**NGS sequencing and analysis:** The libraries will be sequenced on an Illumina Genome Analyzer IIx at the University of Washington on a fee-for-service basis. We have used this facility for RNA-seq from several *Leishmania* species, as well as whole genome NGS and ChIP-seq. The resulting sequence files (in fastq format) will be entered into an RNA-seq pipeline developed by the Seattle BioMed Bioinformatics Core (which is directed by Dr. Myler). Alignment of the reads with the genome is routinely performed using BOWTIE [32], and we have developed scripts to quantitate the number of reads per mRNA. This will be facilitated by the recent studies mapping the 5' and 3' ends of most *T. brucei* mRNAs [11]. Dr. Myler has recently generated similar 5' and 3' mRNA mapping data for *L. major*, *L. donovani* and *L. braziliensis* (see **Fig. 3**). Comparison of read density for individual mRNAs between the randomly-cleaved total and ribosome-associated RNA-seq libraries will reveal whether a sub-set is preferentially excluded from, or recruited to, ribosomes. This will be particularly interesting in the case of RNAs that contain only small ORFs, which are currently the subject of debate as to their function.

Comparison of individual mRNA read densities between the randomly-cleaved and ribosome-footprinted RNA-seq libraries will identify genes that are more efficiently expressed per mRNA molecule within the procyclic stage, thereby providing new information on translational efficiency. We will also

assess the distribution of ribosomes along the mRNA sequence to identify the exact location of the protein-coding sequence within each mRNA and demonstrate whether they are, indeed, translated. This is important because the state of *T. brucei* genome annotation still suffers from uncertainty as to whether the current gene prediction is too low or too high and exactly which protein sequences are expressed by the parasites. We will be able to eliminate some ORFs as non-coding and also to identify new ORFs that may have missed detection because they are quite short or use non-canonical start codons. In yeast and humans, short ORFs upstream of CDSs function in translational control of downstream ORFs on the same transcript [33]. The work will likely require new scripts and pipelines, but we will be guided by the approaches described by Ingolia *et al*. [24]. Our Bioinformatics Core employs several experienced programmers who, under the direction of Dr. Myler, have already adapted and developed a number of pipelines for a variety of purposes, including RNA-seq.

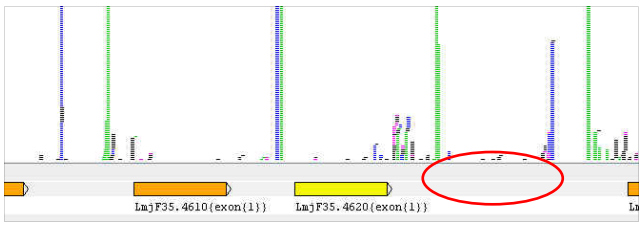


**Figure 3.** RNA-seq analysis of *L. major*
This screenshot shows RNA-seq reads from SL- (green), oligo-dT- (blue), random hexamer-(magenta) and Not-So-Random hexamer- (black) primed libraries prepared from *L. major* Friedlin log-stage promastigotes aligned to a region of *L. major* chr35. The height of each column reflects the number of reads at each position (some are off-scale). Protein-coding ORFs (CDSs) are indicated by orange and yellow boxes. We have effectively mapped SL and polyA sites for almost all genes, but the random- and NSR-primed reads show considerable sequence bias (and were heavily contaminated with rRNA). The red oval highlights an RNA (with SL and polyA tail) containing no annotated CDS and only short ORFs.

The regions protected by the ribosome in yeast began at 12-13 nt upstream of the start codon, presumably representing newly assembled ribosomes at the initiation codon. This could be potentially problematic if the 39-nt SL sequence common to the 5′ end of all trypanosomatid mRNAs were attached within this region, since it would be difficult to map these protected fragments to specific genes. However, only ~5-8% of all *T. brucei* genes have the SL acceptor site closer than 10-nt from the putative initiation codon [11]. The remaining mRNAs should be readily footprinted, even if they are stalled at the initiation site (*i.e.* they are not being translated).

**Aim 2. Identify genes that are regulated at the level of translation during *T. brucei* development.**

The analyses described above will be expanded to bloodstream stages of *T. brucei*, allowing for the comparison of translation regulation within and between stages. Using the pleiomorphic strain 927 provides the opportunity to compare the rapidly-dividing slender bloodstream forms and non-dividing stumpy bloodstream forms stages, which exist within the mammalian host during natural infections, with the procyclic stage characterized in Aim 1. For each stage, three biological replicates will be used, and for each two technical replicates will be employed. The optimized protocols from Aim 1 will be used here to generate ribosome profiles in the two bloodstream stages and the bioinformatics pipeline developed for Aim 1 will be employed to analyze the results of the RNA-seq libraries generated.

**Biological materials:** Slender and stumpy 927 strain bloodstream forms will be obtained after injection into rats. Generally, we obtain $5\times10^8$-$10^9$slender forms and 2-$5\times10^9$ stumpy forms from a single rat. Slender form populations usually have <5% contamination with morphologically intermediate or stumpy forms; while stumpy populations are usually >75% morphologically stumpy, with the remained being predominantly intermediate forms. Morphological characterization will be supplemented with analysis of the ability to express the procyclic-specific molecule procyclin following a shift in medium and temperature (stumpy forms are competent to do so, while slender forms are not). Should parasite numbers be inadequate, we will pool the parasites from individual animals. As a backup we can turn to *in vitro*-derived slender bloodstream forms (one liter of culture yields ~$10^9$ log phase parasites). Our previous microarray analysis [9] demonstrated that *in vitro* and *in vivo* slender bloodstream forms showed little, if any, difference in mRNA abundances. As a final, but unlikely, backup, we could use the monomorphic

strain 427. This strain yields high slender parasitemias *in vivo*, but since monomorphic strains do not recapitulate the natural infection process, they are less desirable for these studies.

**Analysis**: Libraries will be generated and sequenced as described above, if possible using the SL library approach to assess mRNA abundance in the total mRNA and undigested ribosomal fractions, since this would be less demanding and will allow more material to be used for the ribosome profiling libraries. The data from the three developmental stages will then be compared on a gene-by-gene basis to determine whether transcripts corresponding to individual genes differ in representation of ribosome occupancy (*i.e.* they are translated at different rates in different stages). We will determine the relationship between any changes in translation efficiency and differences (or no change) in mRNA abundance. These data will provide information on the role of translational control across different stages of parasite development. As internal controls we will examine transcripts proteins that we and others have already identified as being subject to stage-specific translational control. Additionally, the same types of analyses described in Aim 1 for procyclic forms (*i.e.* searching for evidence of translation for the newly identified putative ORFs and non-canonical start codons) will also be conducted, since it is possible that some of these ORFs are only translated in a particular stage. It is likely beyond the scope of this project to identify the elements within mRNAs that mediate the translational controls, but the data we generate will provide the entrée for such studies in the future.

At present, measuring ribosome occupation of mRNAs provides the most advanced means of assessing translational potential. However, we remain cognizant that these measures are still one step away from measuring protein synthesis and that protein abundance also reflects turnover. In future studies , or as time permits, we will confirm our findings by examining the synthesis of 2-3 proteins that are derived from transcripts showing evidence of stage-specific translational control, utilizing reagents obtained from our previous and on-going projects or from other investigators in the field.

**Data availability:** This project will generate data that will be beyond our capacity to fully exploit in the near term. It is therefore in the interest of the scientific community that the data be available for others who wish to pursue specific aspects of the findings. The NGS sequence data will be made available to the scientific community by submission to the NCBI Sequence Read Archive, as well as TritrypDB (along with more processed results, such as summaries of read densities between life cycle stages). Genes showing translation regulation will also be annotated in GeneDB and TriTrypDB. Dr. Myler and the Bioinformatics group are part of the GeneDB/TriTrypDB consortium and regularly share data with both these databases.

***The proposed studies take a global approach to understanding the relative importance of translational control to stage-regulation of protein expression in* T. brucei. *In addition to this key goal, they will increase our knowledge of the genome capacity of the parasite by identifying which ORFs are translated, and by possibly identifying new, non-canonical ORFs. Should translational control be revealed as an important regulator of protein abundance (as we predict), future studies can address identification of the molecules mediating this control. The proposed experiments will also lay the groundwork for similar studies in other trypanosomatids, such as the various* Leishmania *species and* T. cruzi.**

**Laboratory hazards**: This project employs an animal pathogen *Trypanosoma brucei brucei* (TREU 927), which is considered to be identical to the human pathogen *Trypanosoma brucei rhodesiense*, except it lacks a gene that confers resistance to human serum. While *T. brucei* 927 is not expected to be hazardous to laboratory personnel, universal precautions are always observed for bloodstream stage parasites. The Parsons laboratory has worked with bloodstream stage parasites for over 20 years and all staff receive BSL2 training by Seattle BioMed EHS and in-lab training by experienced laboratory members. For those working with infected animals, training at the University of Washington for mouse and rat procedures will be required. Monthly safety notes related to chemical, radioactive, physical, and biological hazards are discussed at lab meetings.

**10. Vertebrate Animals**

1. Description of animals and how they will be used

**Rats**: Rats will be used for the isolation of bloodstream form pleiomorphic trypanosomes. We anticipate that a total of 30 rats will be used over the two years. We use outbred Wistar rats (male) for growing trypanosomes. The animals are obtained from Simonsen or Charles River Laboratories and must be certified pathogen free. Rats are infected with trypanosomes intraperitoneally using either cultured *T. brucei* or from stabilates previously generated. Infections are monitored for parasite level in the blood and the stage of parasite development assessed. Trypanosomes are harvested by terminal surgery when the populations meet criteria for the percentage of parasites in the desired developmental stage. In some cases the rats may be immunosuppressed with cyclophosphamide or irradiation according to IACUC approved protocols.

**Mice**: Mice will be used to initiate infections should there be challenges in directly infecting rats. The mice are obtained from vendors such as Charles River Laboratories and must be pathogen-free. We anticipate using 5 mice per year, either Balb/c or Swiss Webster. The parasites are harvested by terminal surgery while the trypanosomes are predominantly in the slender, dividing stage.

2. Justification for use of animals

**Rats**: Growth of large quantities bloodform trypanosomes *in vitro* is a costly alternative, requiring large quantities of fetal calf serum. We anticipate that these numbers of animals will provide sufficient biological material for our studies. Rats are good hosts for the strains of trypanosomes we use. These animals are not costly or in short supply. We have set up *in vitro* systems to reduce our reliance on animals for small-scale production.

**Mice**: Mice will be used only to initiate infections should direct infection of rats prove problematic as it sometimes does with older frozen stocks.

3. Veterinary Care

Rats and mice will be housed in the Seattle BioMed animal facility. Seattle BioMed has a PHS Assurance issued by OLAW and has a USDA Research Registration. The facility is under temperature, light and humidity control. Adequate food, water, and bedding are provided. It is staffed by an attending veterinarian, a vivarium manager, and five technicians. The attending veterinarian spends at least 8-16 hours per month at Seattle BioMed, and a back-up veterinarian is on call, including after hours, weekends and holidays. Animal care staff perform routine husbandry procedures and check animals daily for their condition. Most animals are kept for relatively short periods of time, 30-60 days, and if they appear ill, they are euthanized.

4. Provisions to minimize stress, discomfort and injury

If there are signs of distress, the animal staff alert laboratory personnel and the veterinarian as needed. The veterinarian or animal staff may intervene or recommend euthanasia based on animal welfare concerns. Animals are monitored daily by animal care staff. Following injections with parasites, rodents are monitored for parasitemia beginning on day 2. The animals are anesthetized with ketamine/xylazine mix prior to injections of parasites or terminal surgery. This anesthetic does not appear to interfere with the biological function of the parasites.

5. Euthanasia

Mice are euthanized by $CO_2$ asphyxiation. This method is consistent with the recommendations of the American Veterinary Medical Association. When parasites are harvested from rodents, the animals are sedated with ketamine/xylazine, and the blood is collected during terminal surgery in which the aorta is cut. The diaphragm is then cut to fully ensure death while anesthetized.

## 12. Multiple PI Leadership Plan

Drs. Myler and Parsons will act as the project's Principal Investigators, with Dr. Myler acting as the contact PI for grant reporting purposes. Dr. Myler will provide expertise in bioinformatics, genomics and data analysis, while Dr. Parsons will provide expertise in *T. brucei*, including parasite development, experimental design and troubleshooting. They will work together to supervise the project, including scientific and administrative management and oversight, and manuscript preparation. Each will be responsible for direct supervision of personnel within their individual laboratories, but will hold joint lab meetings (with the personnel working on this project) on a regular basis (at least monthly).

Drs. Myler and Parsons have worked together on a variety of projects for over 25 years. In resolving conflict, their ultimate goal will be solving the scientific questions. The deciding factor in any disagreement on research strategy will be what will generate the most complete and quality data.

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**14. Letter of Support**

Nicholas T. Ingolia, Ph.D., University of California, San Francisco (transferring to Carnegie Institution, Department of Embryology, Baltimore, MD).

University of California
San Francisco

UCSF

Cellular and Molecular Pharmacology

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**UCSF MC 2542**
1700 – 4th Street
Byers Hall, Room 404
San Francisco, CA 94158-2330

[redacted]
tel [redacted]

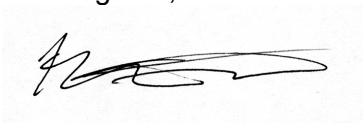
Dr. Peter Myler
Dr. Marilyn Parsons
Seattle Biomedical Research Institute
307 Westlake Ave. N., STE 500
Seattle, WA, 98109

Dear Peter and Marilyn,

I am writing to affirm my interest in your project on ribosomal profiling in the protozoan parasite *Trypanosoma brucei*. I am keenly interested in applying the technology I developed to problems of human health. My understanding is that due to polycistronic transcription, these the pathogens you study likely employ multiple downstream control mechanisms to regulate gene expression. Our technology, which examines ribosome occupancy on mRNAs using a genome-wide approach, will provide clear analysis of the role of translational control in the parasites. It can be used to examine both differences between conditions (i.e., different stages of parasite development) and differences within conditions (i.e., loading of ribosomes onto different transcripts). I have already provided you with our detailed protocol (which also highlights key controls), and will be happy to provide you with additional advice as you move forward in setting up your system.

I will be relocating this fall to establish an independent research program at the Carnegie Institution Department of Embryology, in Baltimore, MD, where my lab will continue to develop the ribosome profiling technology in yeast and metazoa.

With regards,



Nicholas Ingolia

**15. Resource Sharing Plan**

*Data sharing plan*: The resulting gene level data will be provided to public databases such as GEO and TritrypDB.

*Organisms:* not applicable.

*Genome wide studies:* not applicable.

# 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 ☐

**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