PN-ABB-103

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D.C. 20523

DATE: 9/23/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 6, 3, 9
PR. 6/28/88 — 8/28/88

Attachment

6.309

Project Number: 936-5542
Grant Number: DPE-5542-G-SS-7059-00
Project Office: S&T/SCI
Obligation Number: 7361212

This document represents the second progress report for USAID grant #DPE-5542-G-SS7059-00, "Molecular diagnostics for filarial nematodes in mosquito vectors". This covers the second six month (June 28, 1988-August 28, 1988) reporting period.

We have made substantial and significant progress in several areas since the last reporting period. These include development of techniques for isolation and purification of intact mitochondrial DNA from Brugian filariods (after propagation in gerbils), molecular cloning of the complete Brugia pahangi mitochondrial genome, and physical mapping of the mtDNA molecule. Progress in each of these areas is more fully detailed in the following sections:

1. Isolation and purification of intact B. pahangi mtDNA. Microfilaria and adult B. pahangi populations were separately isolated the peritonial cavity of gerbil hosts, washed in sterile Hank's media and stored as wet pellets at -80°C. After thawing, each pellet was resuspended in a mannitol-saline-EDTA buffer and homogenized in a glass tissue homogenizer in the presence of proteinase K. An enriched mitochondrial fraction was prepared from the homogenate by differential centrifugation. Gently resuspended mitochondria were lysed by the addition of sodium dodecyl sulfate, and circular mtDNA isolated from the mitochondrial lysate by ethidium bromide-CsCl isopycnic centrifugation. Alternatively, total cellular DNA obtained by treating nematodes with proteinase K and SDS was fractionated by Hoechst 33258-CsCl centrifugation. By this latter technique, at least five different cellular DNA species were resolved. We have been able to identify three of these species as mtDNA, bulk chromosomal DNA, and ribosomal DNA. We hypothesize that the remaining species represent highly repetitive (satellite) components of the B. pahangi nuclear genome as observed by Wirth and co-workers (McReynolds et al., PNAS 83, 797-801, 1986; Sim et al., Molecular and Biochemical Parasitology 19, 117-123, 1986). Molecular cloning experiments are underway to further identify and characterize these DNA species.

2. Physical characterization of B. pahangi mtDNA. Isolation of intact B. pahangi mtDNA molecules permitted an initial characterization of the mitochondrial genome by restriction enzyme analysis. We have treated the intact mtDNA with numerous restriction enzymes. The molecular weights of restriction enzyme digestion products produced by individual enzymes summate to approximately 14.7 kilobases, which we take to be the molecular size of the B. pahangi mitochondrial genome. This size is in agreement with the size range (14-19 kilobases) measured for a wide variety of animal mtDNAs, including several nematodes (Hyman, Journal of Nematology 20, in press). Based on the molecular weights determined for fragment products resulting from treatment of mtDNA with restriction enzymes individually or in pairs, these fragments could be aligned into a physical map. We have unambiguously positioned 15 restriction sites representing 7 individual enzymes on the B. pahangi mtDNA map. This restriction map will become further refined upon detailed analysis of cloned mtDNA fragments which have recently become available (following section).

3. Molecular cloning of B. pahangi mtDNA. We have successfully cloned the entire B. pahangi mitochondrial genome in the bacterial plasmid vector pUC119. The genome has been cloned as two separate XbaI fragments with lengths of approximately 9 and 6 kilobases. We are currently using these cloned fragments to confirm the restriction map and place new restriction sites within the mtDNA. Detailed restriction maps will be essential designing cloning strategies for efficient diagnostic probe construction. In addition, because pUC119 was chosen as the cloning vehicle, DNA sequencing studies can be performed directly on our cloned material.

4. Future studies during the upcoming funding period(s). The goal of the experiments funded under this proposal is to develop unambiguous diagnostic hybridization probes for B. pahangi and B. malayi, and to use these probes for detection and epidemiological analysis in arthropod vectors. Development of fine structure restriction maps and molecular cloning of the respective mitochondrial genomes are essential initial steps in this process. After developing procedures for the isolation of filariod mtDNA in optimal yields, we have essentially completed the necessary analysis of the B. pahangi mitochondrial genome. We must now carry out essentially the same type of experimentation for B. malayi mtDNA during the next funding period. To this end, 24 B. malayi infected gerbils have been ordered (documentation attached), and are expected to arrive this Fall. Isolation, physical mapping and molecular cloning of B. malayi will be conducted along with simultaneous refinement of the B. pahangi mtDNA restriction map.

When comparative fine structure restriction maps are available, we will then be in a position to assess potential regions of nucleotide divergence between the two mitochondrial genomes as identified by loci exhibiting restriction site polymorphism. These regions will be isolated from the remainder of the mtDNA by molecular cloning and tested for cross-hybridization. The nucleotide sequence of these same regions will be determined so that diagnostic oligonucleotides might be synthesized. During this period, we will begin to use these molecules for detection and identification of nematode mtDNA within arthropod vector lysates using nucleic acid hybridization.

5. Involvement of Dr. Sivakanesan (designated international collaborator) in this project. I have had extensive contact via mail (documentation attacted) with Dr. Sivakanesan regarding collection and shipment of potentially useful samples, including alternate populations of B. pahangi propagated in Jirds. These populations will be extremely useful from an evolutionary standpoint and perhaps provide us with novel mtDNA loci exhibiting restriction site polymorphism. Now that the molecular work is well underway, Dr. Sivakanesan has agreed to visit our laboratory this Fall, where he will become involved with characterization of B. malayi mtDNA. Until cloned diagnostic probes are available, it will be of little scientific use to visit Sri Lanka for the purpose of using these molecules in the field. It is for this reason, along with some confusion regarding the physical status of the Sri Lanka laboratory (see attached letters), that I have not yet made plans to visit Dr. Sivakanesan's facilities.

Dept. of Biochemistry,
Faculty of Medicine,
Peradeniya, SRI LANKA.

1988.02.19.

Dr. Bradley Hyman,
Dept. of Biology, Riverside,
California, 92521, U.S.A.

Dear Dr. Hyman,

Thank you very much for your letter dated 29,January 1988, which I received this morning. I have just finished my consultation with Prof. S.T. Fernando, Dean, Faculty of Veterinary Medicine & Animal Sciences, who is also happened to be the Professor of Veterinary Parasitology, on the aspect of using his laboratory facilities for our research program. Prof. Fernando is embarking on a Project involvin E-S antigens of Toxocara canis, a project to be funded by the University of Cambrid under the Cambridge link. He is a very senior Professor with many years of researc experience in the field of Parasitic Immunology. I feel that our association with Professor Fernando will further strengthen the program. Professor Fernando stated that a new laboratory for his Molecular Biology program will be ready in June this year.

In one of my earlier correspondence, I mentioned about Prof. S. Dissanayake and his research activities. I am sure Prof. Dissanayake will extend his fullest co-operation towards our project. It is possible, sometimes, that the work could be even carried out in his laboratory. However, Prof. Fernando's laboratory is better equipped and less crowded.

Regarding the proposed visitation to the laboratories, you can come here at any time. I will be most happy to take you around and show you the limited facilities we have. Perhaps, you may think of strengthening the research facilities once you visit our laboratories.

As for the sample collection, are you only interested in filarial arthropod vectors or do you need any filarial parasites, microfilariae etc.? When I was in London School of Hygiene and Tropical Medicine, I used frozen unfixed Onchocerca volvulus nodules, O. gutturosa worms, O. gibsoni nodules and Brugia pahangi from Jirds. Some of these specimens could be still obtainable from Dr. C.D. Mackenzie, if I write to him. He used to travel to U.S.A. very often. So, please let me know. Locally we have plenty of Setaria digitata from cattle.

As for the actual collection of the specimens, could you let me have some details regarding storage, processing etc. If I commence the collection early and process the specimens, you will be in a position to take it with you when you return. I hope, I have given you enough information on which you can work out the future plans regarding the project.

Thanking you,

Yours sincerely,

Dr. R. Sivalanesan.

c.c. Prof. S.T. Fernando,
Dean/Vet. Medicine.

UNIVERSITY OF CALIFORNIA, RIVERSIDE

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DEPARTMENT OF BIOLOGY
RIVERSIDE, CALIFORNIA 92521

June 21, 1988

Dr. R. Sivakanesan
University of Peradeniya
Faculty of Medicine
Department of Biochemistry
Peradeniya, SRI LANKA

Dear Dr. Sivakanesan,

Thank you for your letter of June 2, 1988. I apologize for not corresponding with you sooner. I have just finished an intensive teaching commitment which now permits me time for writing. In addition, the project has now sufficiently advanced since I last corresponded with you, so I feel comfortable about forwarding a brief but complete progress report. I shall first update you on our work. Based on these experimental results, I shall then suggest ways in which I believe we can efficiently implement our collaborative efforts.

In the past few months we have made important strides in perfecting methodology necessary for isolating high molecular weight DNA, and intact mitochondrial DNA, from Brugia pahangi. These techniques can be applied to individual nematodes as well as to bulk DNA preparations obtained from nematodes available in large quantities. We obtain B. pahangi directly from infected gerbils that have been purchased from our National Institutes of Health. We incubate nematodes at high temperature in buffers containing proteinase K and SDS. This lysis procedure gently releases total cellular (nuclear and mitochondrial) DNA, which we then purify by a variety of strategies.

Recently, we have fractionated total cellular B. pahangi DNA on CsCl-bisbenzimide gradients. With both insect and plant parasitic nematodes, we generally observe two DNA bands in these gradients corresponding to nuclear and mitochondrial DNA. To our surprise, we observed at least six different B. pahangi DNA species. I suspect that bulk nuclear DNA, mitochondrial DNA and ribosomal DNA can be assigned to three of these bands. Perhaps the others involve highly repeated "satellite" DNA that has recently been observed within the genomes of Brugian filariods. We are actively pursuing the nature of all of these DNAs in our laboratory by a combination of molecular cloning and DNA transfer-hybridization experiments.

With respect to samples which you have offered to make available, we would be happy to study any Brugia, Onchocerea or Wucheraria isolates that you may have at your disposal. We can perform analysis from one or very few nematodes using the cloned probes that we are currently developing (as described above), or we can try to isolate bulk DNA if enough animals can be sent. The nematodes can be freeze dried or stored in ethanol for transit. We do not require live specimens. The possibility of obtaining B. pahangi samples (from Jirds), as you mentioned, sound particulary interesting.

Unfortunately, my schedule does not permit me to visit Sri Lanka this summer. However, I will be in Kyoto, Japan from August 19-27, delivering an address regarding our plant nematode mitochondrial DNA work at the International Plant Pathology Congress. Perhaps you, Dr. Dissanayake or Dr. Fernando would consider the possibility of meeting me there for further discussions regarding our project. I would immediately seek approval from USAID for use of existing travel funds on the grant budget to be used for this purpose. Alternatively, perhaps one of you would consider traveling to my laboratory in Riverside, California to directly participate in B. pahangi mitochondrial DNA isolation. If a molecular laboratory is now in the process of being set up in Peradeniya, as your letter indicated, visitation at this time might provide an invaluable opportunity to see how we have organized such a facility. Funds for travel of this sort have already been approved by USAID.

Again, I apologize for not writing sooner. However, this was a particularly appropriate time to correspond given our recent successes in DNA isolation and characterization. I promise to be prompt with my letters in the coming months. I look forward to hearing from you regarding samples and your interest in either of the trips that I have proposed.

Sincerely,

Bradley C. Hyman

Bradley C. Hyman, Ph.D.
Assistant Professor of
Biology

Dept.of Biochemistry
Faculty of Medicine
Peradeniya
1988.07.04.

Dr. Bradley C.Hyman
Asst.Professor of Biology
University of California
California 92521.

Dear Dr.Hyman,

Thank you for your letter of June 21,1988. It is interesting to note that you have been successful in your initial attempts in the fractionation of DNA from B.pahangi.

Regarding the parasite material,I am writing to Dr.C.D.Mackenzie today.Unfortunately for two of my previous letters Dr.Mackenzie has failed to respond. In his last letter,written to me in January 1988,he mentioned about his contract at the London School of Hygiene and Tropical Medicine(LSHTM) coming to an end in March 1988 but he would maintain contact with me. His silence over the past few months makes me worried. Anyhow,I have already written to his research technician who is still at LSHTM.

Prof.S.Dissanayake's laboratory facilities will not be available for our project till end of this year. He suddenly left the Department and had requested for sabbatical leave till end of 1988.

As you have indicated that your schedule does not permit you to visit Peradeniya this summer,it may be possible for me to visit your laboratory during the University long vacation. As you may be aware that the Peradeniya Campus remains closed from November 1987 and attempts are now underway to get it reopened. So I am not sure when the long vacation is likely to be. But it is very likely to be during August/September.

I will write to you again once I hear about Dr.Mackenzie. In the mean time I will contact Prof.S.T.Fernando regarding the laboratory facilities.

Thanking you.

Yours sincerely,

Sivakanesan

Dr.R.Sivakanesan.

UNIVERSITY OF CALIFORNIA, RIVERSIDE

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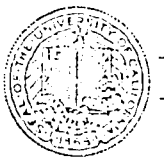

SANTA BARBARA • SANTA CRU

DEPARTMENT OF BIOLOGY
RIVERSIDE, CALIFORNIA 92521

July 20, 1988

Dr. R. Sivakanesan
Department of Biochemistry
Faculty of Medicine
University of Peradeniya
Peradeniya, SRI LANKA

Dear Dr. Sivakanesan,

Thank you for your letter of July 4, 1988, which arrived yesterday. I am quite excited about the possibility of you visiting my laboratory during August or September. I remind you that I will be attending the International Plant Pathology meetings in Kyoto, Japan from August 18-September 3, so a visit in September would be most opportune. Please let me know of your plans so that I might make lodging arrangements for you. Of course, you are welcome anytime it is convenient, other than the two weeks I am away.

I am most sorry to hear that samples from London may not be easily acquired. However, any samples that you may provide us with would be most helpful, as I discussed in my letter of June 21, 1988.

Again, I am most excited about the possibility of your visit and participating in molecular cloning of Brugia mitochondrial DNA.

Sincerely,

Bradley C. Hyman

Bradley C. Hyman, Ph.D.
Assistant Professor of Biology

BCH:gl

Dept of Biochemistry

UNIVERSITY OF PERADENIYA,
FACULTY OF MEDICINE
SRI LANKA
Tel. 08-88315, — Ext. Head 34, Office 35.
08-88301—5 Ext. 350

1988.09.01.

Dr. Bradly Hyman,
Dept. of Biology,
University of California,
USA.

Dear Dr. Hymen,

I hope you will be back by now from Japan. Since I last wrote to you, my attempts to contact Dr. Mackenzie continued and for my luck I received a letter from him, while he was in Australia, indicating his intention to visit Sri Lanka. Subsequently, he informed me from London about receiving my letters and that he will look into my request for parasite material. So there are positive signs from him and I am very much happy about it.

The department of Parasitology in Peradeniya has a breeding colony of Jirds and the Head of the department has given me the full freedom to infect them with B. pahangi. I have written to Dr. Mackenzie to bring the infective material and to establish an infected colony of Jirds. I do hope that my letter will reach him in time. I am also exploring the possibility of obtaining B. pahangi from cats that are maintained in the department of Parasitology, Colombo. When Dr. Mackenzie is here, I will discuss with him regarding B. pahangi from Jirds (London) and other materials at his disposal.

Our Campus is scheduled to re-open on the 5th of September. As such, I have decided to visit your laboratory around late November, when we are likely to be in vacation. Would it be possible for you to proceed with the necessary formalities connected with my visit? I need the President's approval to leave the country and this usually takes two months.

Furthermore, obtaining visa from the American Embassy is becoming difficult at present and a formal letter of award from USAID will make it easy. I prefer if all arrangements are made through US Embassy in Colombo, which will make my visit/training programme official.

Thanking you,

Yours sincerely,

Sivakanesan

Dr. R. Sivakanesan.

Please indicate in the letter the duration of my stay in USA.

REQUISITION FOR RESEARCH MATERIALS

Page _____ of _____

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DR. STEPHANIE L. JAMES
PARASITOLOGY PROGRAM OFFICER
PARASITOLOGY & TROPICAL DISEASES BRANCH
BUILDING: WW, ROOM 737
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

RETURN TO:

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20205

2. RESPONSIBLE INVESTIGATOR (Name, Institution, Dept., City, State)

DR. E.G. PLATZER
DEPARTMENT OF NEMATOLOGY
UNIVERSITY OF CALIFORNIA
RIVERSIDE, CA 92521

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☐ DEL ☐ COLLECT ☐ OTHER
☐ _____ # Returnable Container

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CHAIRMAN, DEPT. OF NEMATOLOGY

4. GRANTING AGENCY

USAID

5. GRANT CONTRACT NO.

DDE-5542-G-SS-7059-00

14. APPROVED FOR SHIPMENT

6. DATE OF REQUEST

AUG. 22, 1988

15. DATE OF SHIPMENT

NO. OF ITEMS (2)	CATALOG OR OTHER NUMBER (3)	DESCRIPTION (9)	16. NUMBER OF ITEMS a. AUTH.	b. SHIPPED
24		GERBILS INFECTED INTRAPERITONEALLY WITH		
		BRUGIA MALAYI		
		DELIVERY BY MID-OCTOBER.		
		COPY OF CALIFORNIA DEPARTMENT OF FISH AND		
		GAME PERMIT ENCLOSED		

10. REMARKS

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892
Building :
Room :MW
(301) 496- 737
2544

August 29, 1988

Dr. E. G. Platzer
Department of Nematology
University of California
Riverside, CA 92521

Dear Dr. Platzer:

We have received your request for filarial material from the University of Georgia. This material is available for research purposes only, through a contract funded by this office.

Your requisition has been approved and forwarded to the University of Georgia and you should receive the materials in several weeks.

We hope that you will find these materials useful in your research. The acknowledgment of this source may be made in any scientific articles resulting from this research as follows: "Materials used in this study were provided by an NIAID supply contract (AI #02642), U.S.-Japan Cooperative Medical Science Program."

Sincerely yours,

Stephanie L. James

Stephanie L. James, Ph.D.
Parasitology Program Officer
Parasitology and Tropical
Diseases Branch
National Institute of Allergy
and Infectious Diseases

UNIVERSITY OF CALIFORNIA, RIVERSIDE

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DEPARTMENT OF BIOLOGY
RIVERSIDE, CALIFORNIA 92521

September 16, 1988

6.309

Re: Grant #DPE-5542-G-SS-7059-00
Project #936-5542

To Whom It May Concern:

Enclosed please find the requested number of copies regarding the FINANCIAL REPORT for the the grant referenced above. This report covers the second six month reporting period (February 28, 1988-August 28, 1988). Feel free to contact me at 714-787-5911 if you have any additional questions.

Sincerely,

Bradley C. Hyman

Bradley C. Hyman, Ph.D.
Assistant Professor of Biology

Rec'd in OC SEP 23 1988

Project Number: 936-5542
Grant Number: DPE-5542-G-SS-7059-00
Project Office: S&T/SCI
Obligation Number: 7361212

6, 30 9

FINANCIAL REPORT:

USAID Grant #DPE-5542-G-SS-7059-00, "Molecular Diagnostics for Filarial Nematodes in Mosquito Vectors." This report covers the second six-month (February 28, 1988-August 28, 1988) reporting period:

1. General assistance, otherwise defined as salaries (benefits not included):

Includes my summer salary and full time technician's salary for Kathryn Weiss, who has worked on plant and insect nematode mtDNA projects in my laboratory prior to transferring to this project.

$6098 spent during this reporting period

Balance remaining in this category is $41469.

2. Supplies and expendibles (S&E):

Costs include chemical and microbiological supplies, laboratory plasticware, gerbils and animal care.

$2025 spent during this reporting period

Balance remaining in this category is $14179.

3. Equipment:

$0.00 spent during this reporting period.

Balance remaining in this category is $591

4. Travel:

Costs include approved travel to the annual Society of Nematologists meeting in Raleigh, North Carolina this past June. Additional funds to be used include one domestic meeting and international travel for myself and Dr. Sivakanesan of Sri Lanka, my international collaborator. These trips are currently being arranged.

$528 spent during this reporting period.

Balance remaining in this category is $10838

Rec'd in SCI SEP 2 3 1988

12

5. Employee benefits (in accordance with category #1 above).

   $2258 spent during this reporting period.

   Balance remaining in this category is $12572

Total spent during this reporting period:	$10909
Total spent during first two reporting periods:	$27399
Total balance remaining on grant:	$79651

(Does not include deductions for university overhead; all figures as of 9/13/88)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892
Building : WW
Room 737
(301) 496- 2544

August 29, 1988

Dr. E. G. Platzer
Department of Nematology
University of California
Riverside, CA 92521

Dear Dr. Platzer:

We have received your request for filarial material from the University of Georgia. This material is available for research purposes only, through a contract funded by this office.

Your requisition has been approved and forwarded to the University of Georgia and you should receive the materials in several weeks.

We hope that you will find these materials useful in your research. The acknowledgment of this source may be made in any scientific articles resulting from this research as follows: "Materials used in this study were provided by an NIAID supply contract (AI #02642), U.S.-Japan Cooperative Medical Science Program."

Sincerely yours,

Stephanie L. James

Stephanie L. James, Ph.D.
Parasitology Program Officer
Parasitology and Tropical
Diseases Branch
National Institute of Allergy
and Infectious Diseases

14

REQUISITION FOR RESEARCH MATERIALS

Page _____ of _____

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BETHESDA, MD 20892

RETURN TO:
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20205

2. RESPONSIBLE INVESTIGATOR (1-27) (Name, Institution, Dept., City, State)
DR. E.G. PLATZER
DEPARTMENT OF NEMATOLOGY
UNIVERSITY OF CALIFORNIA
RIVERSIDE, CA 92521

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CHAIRMAN, DEPT. OF NEMATOLOGY

4. GRANTING AGENCY
USAID

5. GRANT/CONTRACT NO.
DDE-5542-G-SS-7059-00

6. DATE OF REQUEST
AUG. 22, 1988

14. APPROVED FOR SHIPMENT

15. DATE OF SHIPMENT

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NIH 381-2 (Rev. 5/84)