Bases génétiques et moléculaires des interactions de la cellule eucaryote

Rapport Hcéres

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

AERES report on the research unit

Bases Génétiques et Moléculaires des Interactions de la Cellule Eucaryote

From the

Pasteur Institute

CNRS

May 2010

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

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From the

Pasteur Institute

CNRS

Le Président
de l'AERES

Jean-François Dhainaut

Section des unités
de recherche

Le Directeur

Pierre Glorieux

May 2010

Research Unit

Name of the research unit: Bases génétiques et moléculaires des interactions de la cellule eucaryote

Requested label: URA CNRS

N° in the case of renewal: URA 2581

Name of the director: M. Arthur SCHERF

Members of the review committee

Committee chairman

Ms. Alistair CRAIG, Liverpool, UK

Other committee members

M. David ROOS, Philadelphia, USA

M. Graham BROWN, Parkville, Australia

Ms. Deborah SMITH, York, UK

M. Mike FERGUSON, Dundee, UK

M. Neil GROW, Aberdeen, UK

M. José RIBEIRO, Bethesda, USA

M. Mats. WAHLGREN, Stockholm, Sweden

M. Michel NUSSENZWEIH, NY, USA

M. David SIBLEY, St. Louis, USA

Committee members suggested by CNU, CoNRS, CSS INSERM, CSS INRA, INRIA, IRD

M. Jérome ESTAQUIER, CoNRS

Observers

AERES scientific advisor

M. Nicolas GLAICHENHAUS

University, School and Research Organization representatives

Ms. Evelyne JOUVIN-MARCHE, CNRS (not present at the date of the visit)

M. Alain ISRAEL, Pasteur Institute

Report

1 • Introduction

- Date and execution of the visit :

This unit was evaluated as part of the Department of Parasitology and Mycology on October 7, 2009.

- History and geographical localization of the research unit, and brief presentation of its field and scientific activities :

This unit belongs to the Department of Parasitology and Mycology of the Pasteur Institute.

- Management team :

The head of this unit is M. Arthur Scherf.

- Staff members (on the basis of the application file submitted to the AERES) :

N1: Number of researchers with teaching duties (Form 2.1 of the application file)	0
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	5
N3: Number of other researchers (Form 2.2 and 2.4 of the application file)	10.5 + 17
N4: Number engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	12
N5: Number engineers, technicians and administrative staff without a tenured position (Form 2.6 of the application file)	0
N6: Number of Ph.D. students (Form 2.7 of the application file)	12
N7: Number of staff members with a HDR or a similar grade	3

2 • Overall appreciation on the research unit

- Summary

This unit has enabled the success of several scientific projects (see below), including the establishment of a new unit of Trypanosome Cell Biology, and the nucleation of additional funding from the Gates Foundation and elsewhere.

The recent reorganization to focus exclusively on parasite biology makes programmatic sense.

Notwithstanding the impressive achievements of some groups, the URA has yet to become more than the sum of its parts. Limited communication between groups may compromise the ability to capitalize on potential synergies.

The CNRS program has enabled valuable training for young scientists, although more active engagement in mentorship and career guidance might be helpful.

The establishment of a departmental seminar series is a positive step, which could be enhanced through more active participation.

A common proteomics platform has been integral to achievements from the 2 and 3 laboratories, but the potential of common platform for parasite transfection has yet to be realized; both platforms could benefit from greater clarity of cost structure, oversight, and metrics for evaluating performance.

Recruiting additional members to join this group makes sense, and is justified in the context of imminent retirements … provided that the promise of increased departmental communication can be achieved.

- Production results

A1: Number of lab members among permanent researchers with teaching duties who are active in research (recorded in N1 and N2)	0
A2: Number of lab members among permanent researchers with or without teaching duties who are active in research (recorded in N3, N4 and N5)	16
A3: Ratio of members who are active in research among staff members [A1/(N1+N2)]	16 / 16

3 • Appreciation team by team

3 • 1 Appreciation on team # 1:

Title of the team: Biology of Host Parasite Interactions

Name of the team leader: M. Arthur SCHERF

- Staff members (on the basis of the application file submitted to the AERES)

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)	0	0
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	5	4
N3: Number of other researchers (Form 2.2 and 2.4 of the application file)	8	8
N4: Number engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	6	5
N5: Number engineers, technicians and administrative staff without a tenured position (Form 2.6 of the application file)	1	1
N6: Number of Ph.D. students (Form 2.7 of the application file)	4	1
N7: Number of staff members with a HDR or a similar grade	3	2

- Appreciation on the results

One and/or last author original publications during the past 4 years include papers in Cell, 2008 ; Cell Host and Microbe, 2009 ; JEM, 2008 ; Nature Structural and Molecular Biology, 2008 ; Molecular Microbiology, 2007 ; Cellular Microbiology, 2008 ; EMBO reports, 2005 ; J Immunol, 2006 ; PloS one, 2009 and 2007 ; Microbes and Infection, 2008 and 2006.

The team members have also published reviews in Cell (2006), Annual Review of Microbiology (2008), and Med Sci (2009).

- Strengths

-One of the world's leading laboratories in the field of antigenic variation in malaria (as numerous international references attest); excellent publication record in leading international journals;

-Effective implementation of cutting-edge technologies (FISH, ChIP, etc);

-Good interactions within the group, including scientific mentoring of junior staff;

-Proposed future directions are novel and exciting;

-Leadership role in the BioMalPar Network of Excellence (EU FP6/7) has had an extraordinary impact on malaria research throughout Europe, drawing considerable attention to the department as a whole.

- Weaknesses

-The objectives of activities unrelated to antigenic variation and var gene silencing are not well defined;

-Support for pathophysiology is relatively weak within the group; the affiliated project on immune inflammation is an awkward fit, and has not been particularly productive;

-The transgenic platform has yet to achieve cost-effectiveness, and criteria for evaluating performance (number of constructs, cost per transgenic, service to other groups, etc) have not been defined;

-Limited communication with other groups in the department is a disadvantage for all concerned.

- Recommendations

-Studies on antigenic variation are world-class, and should continue to be supported at a high level;

-Evaluation of junior scientists as potential group leaders should consider goal: development of independent research teams, or building upon the excellence of the current group;

-Define cost structure for the transfection technology platform, implement an oversight committee and a platform user group, and define metrics for evaluating performance.

3 • 2 Appreciation on team # 2:

Title of the team: Provisional unit of Trypanosoma Cell Biology

Name of the team leader: M. Philippe BASTIN

- Staff members (on the basis of the application file submitted to the AERES)

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)	0	0
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	1	1
N3: Number of other researchers (Form 2.2 and 2.4 of the application file)	3	3
N4: Number engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	1,3	1,3
N5: Number engineers, technicians and administrative staff without a tenured position (Form 2.6 of the application file)	0	0
N6: Number of Ph.D. students (Form 2.7 of the application file)	3	3
N7: Number of staff members with a HDR or a similar grade	1	1

- Appreciation on the results

First and/or last author original publications during the past 4 years include papers in Journal of Cell Science, (2009, 2008, 2006), Cellular Microbiology (2009) ; PloS one (2007); Molecular Biology of the Cell (2008) ; Molecular and Biochemical Parasitology (2007), BMC biotechnology (2005).

- Strengths

-Clear thinking, dynamic scientist, internationally recognized by the trypanosome molecular parasitology community, and increasingly by ciliary disease biologists. The committee received numerous positive evaluations from external referees, attesting to the high regard in which the PI is held by parasitology researchers.

-Clever experimental design, including impressive purification of flagella, and multidisciplinary collaboration with physicists to develop real-time statistical imaging of intraflagellar transport.

-Impressive mentorship abilities, manifested in outstanding esprit de corps.

- Weaknesses

-Publication record is adequate, but not yet outstanding (although on a positive trajectory);

-The field of trypanosome flagellar biology is highly competitive, and may be saturated … although the team leader is (more than) holding his own;

-Future plans are exciting, although perhaps too broad and betraying some naïvete regarding quantitative proteomics;

-Success may be compromised by inadequate access to standard spinning-disk confocal microscopy, and the surprising lack of SILAC technology at Pasteur.

- Recommendations:

-Continue as a full unit. Strong support for the requested addition of a technician; support for an additional postdoc may be justified, although proposed project was not clearly defined;

-Establish a small facility for rearing tse-tse flies and vector-borne infection of mice, enabling important studies on trypanosome development in the fly and at the site of infection;

-Ensure adequate access to spinning-disk confocal microscopy (at least 50%), which is essential for all studies on intraflagellar transport;

-Provide access to LC-MS/MS facilities and software for SILAC quantitative proteomics. (Other groups throughout the Insititut will also benefit from this equipment.);

-Providing access to external reviews (appropriately redacted) would be helpful in devising future plans.

3 • 3 Appreciation on team # 3:

Title of the team: G5 group of Parasite virulence

Name of the team leader: M. Gerald SPAETH

- Staff members

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)	0	0
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	2	2
N3: Number of other researchers (Form 2.2 and 2.4 of the application file)	2	2
N4: Number engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	3	3
N5: Number engineers, technicians and administrative staff without a tenured position (Form 2.6 of the application file)	3	3
N6: Number of Ph.D. students (Form 2.7 of the application file)	2	2
N7: Number of staff members with a HDR or a similar grade	1	1

- Appreciation on the results

First and/or last author original publications during the past 4 years include papers in PLoS Pathogens (2009) ; Cellular Microbiology (2009) ; Experimental Parasitology (2008) ; Proteomics (2008) ; International Journal for Parasitology (2007).

- Strengths

-In vitro analysis of phosphoproteins in Leishmania is an important advance, although it will be necessary to evaluate the significance of these findings in vivo

-The LeishDrug network led by Späth promises collaborative and synergistic interactions with others, and may advance Leishmania kinases as bone fide drug targets.

- Weaknesses

Some of the proposed in vitro approaches are prone to identifying permissive targets of kinases that may not be valid in vivo. Strict criteria should be established for evaluating and prioritizing hits to be pursued (and discontinuing less promising leads) ;

The project is somewhat diffuse and perhaps overly ambitious. For example, it is not clear that significant progress can be made on both the MAP kinases and new kinases discovered by in-gel methods. (The latter seems particularly risky, as hints of success will inevitably lead to the identification of kinases impacting other pathways, further detracting from the focus of the project.)

Newer proteomics methods based on LC-MS/MS offer improved resolution and precision relative to 2D gels; exploiting such approached would enhance the project.

- Recommendations

-It is essential that work now in progress is seen into print as rapidly as possible

-Focus on identifying targets and regulation of the MAPK pathway, and defining its role in differentiation

-The potential impact of this group's research could be enhanced considerably by developing conditional genetic mutants, and implementing newer approaches for quantitative proteomics (see above).

3 • 4 Appreciation on team # 4:

Title of the team: Parasite Immunology

Name of the team leader: Odile PUIJALON

- Staff members

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)	0	0
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	2	2
N3: Number of other researchers (Form 2.2 and 2.4 of the application file)	2	3
N4: Number engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	0	0
N5: Number engineers, technicians and administrative staff without a tenured position (Form 2.6 of the application file)	4,5	4,5
N6: Number of Ph.D. students (Form 2.7 of the application file)	3	2
N7: Number of staff members with a HDR or a similar grade	2	3

- Conclusion :
  - Strengths

This group exploits exceptional knowledge on the biology of P. falciparum to study the complex interaction with the spleen, suggesting that sub-populations of ring-infected erythrocytes are removed upon splenic passage

A novel in vitro method using a heterogenous metal-matrix to mimic selective RBC removal has been patented, and could potentially be important for ex vivo removal of infected erythrocytes in clinical hyperparasitemia or severe malaria

Studies on the role of subtilisins in parasite biology have identified possible inhibitors

Laboratory and field studies suggest possible correlates of artemisinin tolerance

Functional studies have focused on the DBL1a domains of PfEMP1, and the role of RESA in enhancing the stability of infected erythrocyte membranes.

– Weaknesses

-The diversity of research from this group makes it difficult for all findings to be carried forward effectively; work is of variable quality

-Collaboration with other members of the department working on related issues has been limited

-Productivity / visibility / impact is quite modest in relation to overall staffing levels

-It is not clear where future leadership of this group may lie.

– Recommendations

Work on splenic clearance is highly recommended for further development, with greater focus on projects uniquely developed by this team.

Note de l'unité	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A+	A	A+	A	A+

Nom de l'équipe : BIOLOGY OF HOST PARASITE INTERACTIONS

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A+	A+	A+	A	A+

Nom de l'équipe : PROVISIONAL UNIT OF TRYPANOSOMA CELL BIOLOGY

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A+	A	A+	A	A+

Nom de l'équipe : PARASITE VIRULENCE

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	A	A

Nom de l'équipe : PARASITE IMMUNOLOGIE

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	A	A

INSTITUT PASTEUR

Head : Prof. Artur Scherf

Biology of Host-Parasite Interactions Unit

Director : Artur Scherf DR1

URA2581 Bases génétiques et moléculaires de la cellule eucaryote

Paris, 11th May 2010

Subject: Reply to the AERES evaluation report of the URA2581 and the Biology of Host-Parasite Interactions Unit

Dear committee members,
Dear representatives,

First of all, I would like to thank the committee members for having participated in this evaluation process and for their constructive and critical feed back. This is much appreciated by all members of the URA 2581.

URA2581:

We appreciate the very favourable comments on the URA2581 in the AERES report and that the URA has been the nucleation point for the establishment of a new unit on Trypanosome biology. We aim to continuously promote the establishment of new emerging parasitology groups in the future. This is one of our major ambitions.

The complexity of the administrative levels of the URA and individual teams, however, has escaped to a certain extent this commission, which has been composed nearly entirely of international evaluators not familiar with the French system. For example, the four teams of the CNRS URA work at Pasteur in the Department of Parasitology (which has been evaluated by the same commission). In addition, several small teams have been annexed administratively to the larger teams (see team 1). This has led to some confusion in the general recommendations.

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More specifically, the summary AERES report of the URA mentions limited communication between groups. This criticism is justified at the level of the Department of Parasitology and Mycology but not at all relevant for the URA2581. The URA organizes regular seminars with almost all members participating at these meetings demonstrating mutual respect for each other. The coordinated purchase of several equipments and the sharing of parasite culture rooms between members are other examples for the communication between the teams. The URA clearly is a well functioning and internationally recognized ensemble in the Molecular and Cellular Parasitology community.

Biology of Host Parasite Interaction Unit

We are pleased to read that the topics developed in the team 1 and our way of functioning has been appreciated.

The committee members have raised several issues. The first one was about some of our future objectives, which apparently did not appear clear. All future activities of team 1 are already supported by three national (2 ANR and 1 FRM grant) and several international grants (2 Gates Foundation, 2 FP7 malaria network grants). Thus, we feel that we have been able to convince other evaluation committees of the soundness and clarity of our approaches. May be the short time of the presentation was not sufficient to develop fully the potential of our projects.

The second aspect was the relatively weak support for pathophysiology in the group in the future projects. I agree with this point, which is due to the retirement of Juerg Gysin, after more than 25 years of close collaboration. His creative research activities had produced constantly novel discoveries and he is clearly a very big loss for the unit. In addition, Benoit Gamain has reached maturity to develop his own research line on placental malaria. He has received an award (ATIPE) to start his own laboratory in 2010. These two factors contribute to a weakening of the pathophysiology group. Depending on future grants on placental malaria, this research line will be strengthened again by hiring new post docs.

The third concern is the transgenic platform. This platform has been first created at the level of the Department of Parasitology and Mycology at the demand of several malaria groups in order to raise international competition. The overall activity of this platform has been positive after 2 years of existence (although some groups have not fully exploited its possibilities). Given the

high demand for transfection of *P. falciparum* for a new ERC advanced grant project within the URA2581, the platform has been fully integrated into team 1.

Another concern is the communication between teams in the Department of Parasitology and Mycology. This is a major worry for the head of this Department. In the URA2581, however, this is not a concern as has been pointed out at the beginning of this letter.

Again, we would like thank all evaluators for the time spent to look critically into our activities. These comments have been integrated to improve our future research activities and management of the URA2581.

Best regards,

Artur Scherf (DR1 CNRS and Prof. Institut Pasteur)
Responsable for « Biology of Host-Parasite Interactions Unit »
Directeur of the URA2581 « Bases génétiques et moléculaires des interactions de la cellule eucaryote »

Alain ISRAËL
Directeur de l'Evaluation scientifique
Institut Pasteur

Unité postulante de Biologie Cellulaire des Trypanosomes

URA2581 Bases génétiques et moléculaires de la cellule eucaryote

Paris, 9th May 2010

Dear committee members,
Dear representatives,

as requested by the AERES procedure, we are responding to the evaluation letter. First, we would like to thank the committee members for having participated to this evaluation process and for their valuable suggestions. We are happy to read that the topics developed in the group have been appreciated and that our way of functioning has been appreciated.

The committee members have raised three comments. The first one was about the publication outcome described as 'adequate' but 'on a positive trajectory'. We have published three collaborative papers over the last 6 months in well-recognised journals (Journal of Cell Biology, Journal of Cell Science, American Journal of Human Genetics). Moreover, another collaborative work with the team of Gerald Späth (also member of the URA2581) has recently been accepted for publication in PLoS Neglected Tropical Diseases. We have initiated several totally new projects since 2007 whose main achievements have been highlighted by the committee: purification of intact flagella and functional validation, visualisation and quantification of intraflagellar transport, including determination of individual protein complexes in the control of the process and finally identification of novel development steps of trypanosome in the tsetse fly with revelation of a key protein for the life cycle. The outcome of these works are currently being written up and will be submitted in the coming months.

The second aspect was the high level of competition in the flagellum field, although the committee mentioned that the group was holding his rank. We would like to point out that the PI

has been invited to give talks at the two most prestigious meetings in the field in 2010 (Gordon Conference on Host-Parasite Interactions and Faseb conference on Cilia and Flagella), showing international recognition. The final point of concern was the access to imaging and proteomics technology. We have recently secured a grant to acquire a new spinning disk microscope equipped with FRAP and TIRF in collaboration with the imaging platform (headed by Spencer Shorte). We assumed the scientific coordination of the project that involved both parasitology and bacteriology. This grant also includes super-resolution light microscopy (PALM or STORM), an approach that promises a supplementary leapfrog for our projects. Finally, we are also actively involved in proteomics as we drove a parasitology project that allowed the recent acquisition of an Orbitrap LTQ spectrophotometer equipped with an ETD module. This equipment is based at the proteomics platform and has already contributed to novel results for the flagellum purification project.

We are confident that these recent developments should allow us to carry out successfully our research programme.

Thanks again for your thorough work.

Best regards,

Dr. Philippe Bastin (DR2 INSERM)
Responsable de l'unité « Biologie Cellulaire des Trypanosomes »
Directeur adjoint de l'URA2581 « Bases génétiques et moléculaires des interactions de la cellule eucaryote »

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URA2581
Bases génétiques et moléculaires de la cellule eucaryote

Paris, 11th May 2010

Dear committee members and Representatives of the Tutelles,

We would like to thank the committee for the helpful comments. We are pleased to note that the committee valued our expertise and emphasis on parasite biology and pathophysiology as well as endorsed the risks taken by the Unit to explore poorly documented host parasite interactions using unique, yet logistically demanding experimental sets up established in the laboratory. We have taken steps to address the weaknesses noted by the committee and will make all efforts to follow the committee's recommendations. The risks taken over of some projects are now paying off; we have communicated some recent results and are in the process of doing so for other recent ones.

We thank the committee for the recommendations to foster collaborations within the URA and within the Department. Collaborations have been indeed initiated within the URA since the visit and collaborations within the department are actively pursued. As the Department Chair, improving interactions by capitalising on complementary expertise within the Department is my priority.

Yours sincerely

Odile Puijalon

INSTITUT PASTEUR

Gerald Späth, Ph.D.
Chef de Laboratoire
Head, G5 Virulence Parasitaire
Director, FP7 LEISHDRUG consortium

Paris, 05/05/2010

Object: AERES report on the research unit "Molecular and Cellular Host-Parasite Interactions"

Dear committee members, dear representatives,

I want to thank you for time and effort spent to evaluate our team, and I am grateful for your comments and constructive criticisms. I appreciate that the committee recognized the scientific impact of our phosphoproteomics analyses and acknowledged the potential of the FP7 LEISHDRUG network to define *Leishmania* kinases as bone fide drug targets. We are pleased that over the last six months we anticipated the following recommendations given by the committee.

First, the committee recommended that work in progress is seen into print as rapidly as possible. Several manuscripts have been published over the last months or at various stages in the publishing process:

1. Dujardin J.C, González Pacanowska D., Croft S.L., Olesen O.F., and Späth G.F., "Collaborative actions in anti-trypanosomatid chemotherapy with partners from disease endemic areas", Trends in Parasitology 2010, *in press.*
2. Foucher A.L., Späth G.F., and I.K. Pemberton, "Probing the dynamic nature of signalling pathways by IMAC and SELDI-tof MS", Archives of Physiology and Biochemistry 2010, *in press.*
3. Wai-Lok Yau W.Y., Blisnick T., Taly J.F., Helmer-Citterich M., Schiene-Fischer C., Leclercq O., Li J., Schmidt-Arras D., Morales M.A., Notredame C., Romo D., Bastin P., and Späth G.F., "Cyclosporin A treatment of Leishmania donovani reveals stage-specific functions of cyclophilins in parasite proliferation and viability", PloS NTD, 2010, *in press.*
4. Morales M.A., Watanabe R., Dacher M., Chafey P., Osorio y Fortéa J., Scott D.A., Beverley S.M., Ommen G., Clos J., Hem S., Lenormand P., Rousselle J., Namane A., and Späth G.F., "Phosphoproteome dynamics reveal heat shock protein complexes specific to the Leishmania donovani infectious stage", PNAS 2010, Apr 19.
5. Morales M.A., Pescher P., Späth G.F., "Leishmania major LmaMPK7 protein kinase activity inhibits intracellular growth of the pathogenic amastigote stage", Eukaryot Cell. 2010 Jan;9(1):22-30.
6. Hem S., Gherardini P.F., Osorio y Fortéa J., Hourdel V., Morales M.A., Watanabe R., Pescher P., Kuzyk M.A., Smith D., Borchers C.H., Zilberstein D., Helmer-Citterich M., Namane A., and Späth G.F., "Identification of Leishmania-specific protein phosphorylation sites by LC-MS/MS and comparative genomics analyses", submitted.

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Second, the committee recommended to focus on identifying targets and regulation of the MAPK pathway, and defining its role in differentiation. We recently made important progress towards these goals and two manuscripts are preparation. We genetically defined the *Leishmania* MAP kinase LmaMPK7 as stress-regulated protein kinases and identified a highly unusual co-chaperone as a major target (Morales et al., in preparation). Furthermore, we obtained the high resolution structure of LmaMPK10, and revealed regulation of this protein kinase by an auto-inhibitory, parasite-specific C-terminal domain (Schmidt-Arras, in preparation).

Third, the committee noted that the potential impact of this group's research could be enhanced considerably by developing conditional genetic mutants, and implementing newer approaches for quantitative proteomics. Using a novel conditional knock out strategy based on a negative selectable marker, we have established a conditional null mutant for the co-chaperone STI1 and used this parasite line for the analysis of phosphorylation site mutants using a plasmid shuffle approach (Morales et al., PNAS 2010). The same strategy is currently applied on the three *Leishmania* MAP kinase homologs LmaMPK4, 7, and 10, all of which are essential for *Leishmania* survival (and thus could not be targeted by classical knock out strategies). We pursued our phosphoproteomics analysis of *L. donovani* implementing newer approaches and have a first manuscript submitted on the LC-based identification of *L. donovani* phosphorylation sites (Hem et al, submitted). In addition, a detailed time course experiment analyzing changes in phosphorylation pattern during the pro- to amastigote differentiation using quantitative phosphopeptide analysis by iTRAQ-LC-MS/MS has been concluded as part of the FP7 LEISHDRUG project.

Thank you for your support and consideration,

Sincerely,

Gerald F. Späth, Ph.D.

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