Japanese-Swiss Science and Technology Programme

Young Researchers Exchange Programme between Japan and Switzerland
Scientific & Financial Report

Project No.	EG 11-2014
Project title	**Functional analysis of *Toxoplasma gondii* aspartyl protease as potential mediator for the formation of intravascular nanotubular network**
Fellowship period	**1.1.2015-31.03.2015**
Swiss Principal Investigator / Host	University of Geneva/ Prof. Dominique SOLDATI-FAVRE
Japanese Fellow	**KAMEYAMA Kyoko** Research Fellowships for Young Scientists of Japan Society for the Promotion of Science

1. Summary of scientific achievements (max ½ page; incl. pictures, suitable for publication)

The *Apicomplexa* phylum includes important human pathogens such as *Plasmodium* species, the causative agents of malaria, and *Toxoplasma gondii* (*T. gondii*), a common cause of congenital ocular toxoplasmosis and cerebral diseases in immunocompromized individuals. *T. gondii* enters host cells via an active process resulting in the formation of a parasitophorous vacuole (PV), a secluded environment in which the parasite safely resides and replicates. The ability of *T. gondii* to penetrate host cells, subvert their cellular functions and divert their defense mechanisms is facilitated by the presence of specialized secretory organelles, including rhoptries, micronemes and dense granules.

The dense granules have recently been identified as key contributors to the secretion of effector molecules that reach the host cell cytosol or nucleus to hijack host-signaling pathways. The export of dense granule proteins (GRAs) beyond the parasitophorous vacuole membrane (PVM) involves as yet poorly characterized translocon machinery within *T. gondii*, which has however been the focus of intense investigations within *Plasmodium falciparum* (*P. falciparum*) in recent years. A central *P. falciparum* enzyme implicated in cleavage of proteins trafficked across the PVM and into the infected erythrocyte is Plasmepsin V (PfPMV). PfPMV cleaves PEXEL motif-containing substrates in the endoplasmic reticulum (ER) of the malaria parasite and thus uncovers a key signal for trafficking beyond the PV. We propose in this project to undertake a functional characterization of the closely related aspartyl protease 5 in *T. gondii* (TgASP5).

2. Scientific project achievements (max 2 pages)

21. Determine the fate of TgGRA7 in TgASP5 KO parasites

T. gondii GRA7 is a secreted dense granule protein found abundantly in the PV establishing. Previous result showed that GRA7 is phosphorylated in the host cell, and located inside PV, PVM and host cell cytosol. Besides, binding of GRA7 to immunity-related GTPase and led to enhanced polymerization. GRA7 has various functions for parasite survival in the host cell, but control mechanism of secretion and localization is still unknown.

To determine if deletion of ASP5 effect on GRA7 secretion and localization, we infect two strain of Toxoplasma to Human Foreskin Fibroblast cells (HFF) cell. Infected HFF cell by RH strain as a wild type and ASP5 knockout parasite were analyzed by indirect fluorescent assay (IFA) (Figure. 1). The sample with fixation by 4% Paraformaldehyde Phosphate Buffer Solution (PAF) showed no difference between wild type and ΔASP5 parasite. Dense granules of both parasite strains were stained clearly (upper pictures). Interestingly, the result of IFA with glutaraldehyde (PAF/GLU) fixation, compare to wild type parasite, only dense granules were stained in ΔASP5 parasite (lower pictures). Glutaraldehyde allow to fix more deeply, can fix secreted protein in parasite cytosol and vacuolar space of PV. Suggesting that GRA7 was expressed in both parasite straind, but ΔASP5 parasites exhbited an impact on the se GRA7 secreted. Indeed the series of phosphorylation events leading to higher MW isoforms are absent in GRA7 indicating that the protein is not targeted anymore tot he host cell side oft he PVM where the posttranslational modification takes place.

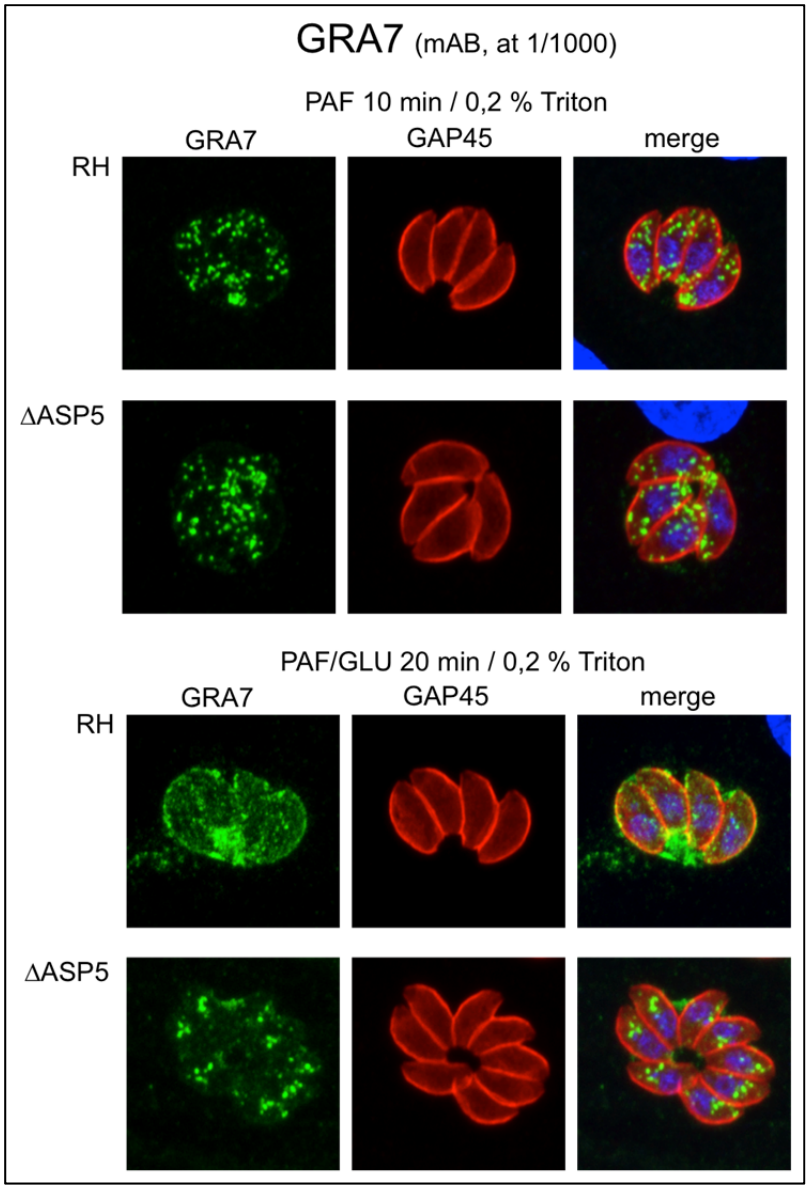
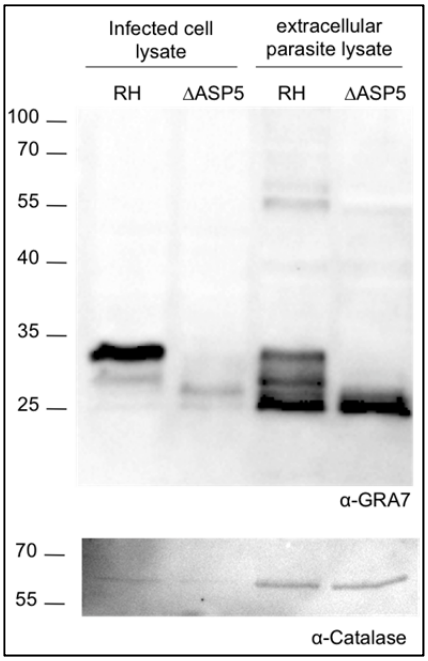


Fig.1 IFA result

Fig.2 WB result



To confirm if GRA7 is degraded by ASP5 or not, we compared protein size in parasite (extracellular parasite) and protein secreted into host cytosol (infected cell lysate) by western blotting analysis (WB). GRA7 has Pexel like motifs (REPLE, RSDAE, RSFKD), but protein decoration mechanism via Golgi body is not excluded yet. From WB result, the size of secreted GRA7 was around 36 kDa, bigger than previous study. We are trying to repeat this experiment again to confirm the result.

2. Determination of the fate of CPC3 in TgASP5 KO parasite

Cysteine proteases are important for the growth and survival of apicomplexan parasites. The parasite expresses five families of cysteine proteases, including three cathepsin C-like (TgCPC1, 2 and 3), one cathepsin L-like and one-cathepsin B-like proteases. Recent studies are revealing that *T. gondii* cathepsins function in microneme and rhoptry protein maturation, host cell invasion, replication, and nutrient acquisition. Specifically, we focused on ASP5 role on trafficking and maturation of TgCPC3.

To analyze the localization of TgCPC3 in *T. gondii,* we generated and confirmed the plasmids for inducing express of tagged TgCPC3. ASP5 lox and ASP5 KO strain parasite were

transfected and selected by bleomysin. However, WB and IFA could not detect the tag of TgCPC3. We hypothesized that TgCPC3 can be digested very quickly after secretion. We are planning to continue analysis with proteasome inhibitor to avoid loss of TgCPC3.

3. Partnership

- Is the exchange based on an existing partnership between the Japanese and Swiss research groups?

No. We had no contact until this program, this exchange opened up both of our laboratories to start collaboration study between Japanese and Swiss research groups.

- Did the cooperation between fellow and host go well?

Yes, the host Prof. Dominique SOLDATI-FAVRE cared me a lot,. I was able to benefit a lot from laboratory reagents and expertise were kind and nice experimental environment. I really appreciate to have a chance to join her laboratory.

- Will there be a continued collaboration after the return home of the Japanese fellow?

Yes, my supervisor and I interest in the project that I did in Swiss under Prof. Soldati-Favre. We will start collaboration study immediately. Exchange of database and experimental tequniques each other must help research of both elaborately.

4. Please describe how the Swiss host and Japanese fellow have benefited from this exchange

From this exchange program, I learned many things.
1. Experimental procedures, which can be utilized in, research activities in Japan. Even the same experimental contents, each laboratory has a slightly different way, so it opens my mind to get answer when I consider new experiment.
2. It changed my mind dramatically by learning in the top lab of research area.
3. Discussion capability in English is improved.
4. All in a new environment and, I learned the importance of moving from their own.
From this exchange, new connection between two laboratories, it may help to develop study each other.

5. Outlook

In Nishikawa lab, in order to evaluate the GRA7 as a vaccine antigen, and it has implemented collaboration with Japanese companies and other laboratories. Therefore, in considering the potential of other GRA proteins derived from *Toxoplasma* as a vaccine candidate, exhaustive analysis result of Prof. Dominique laboratory must become strong helper. It is expected that collaboration study accurate to develop practical treatment of *Toxoplasma*.

6. List of Publications

None.

7. Miscellaneous

- Do you expect any patents coming out of this project?

None.

- Do the results of this project have commercial potential? Do you think there could be an industrial partner involved in this project in the next phase?

None.

8. Suggestions for the next phase of the exchange program

There was not enough time to prepare until exchange start after gets result of this offer.

9. Financial report

- Please include a copy of the account report from your financial department concerning this project. Original receipts need not accompany this financial report. However, please keep the original receipts for 5 years after the project has finished

For details, please refer to the attached sheet.

UNIVERSITE DE GENEVE / SERVICE FINANCIER — Rapport financier final - SITUATION GLOBALE

Edité : 16.06.2015
Page : 1

Fonds..... : ME10061 SER/JST (ETHZ) 2014 SOLDATI-FAVRE DOMINIQUE
Financé par..... : UNIG Fonds institutionnel global
Période..... : 01.01.2015 au 31.05.2015
Centre financier : MIMOL
Requérant(s).... : SOLDATI-FAVRE DOMINIQUE
Allocation totale: 12,000.00

Solde selon précédent rapport : 0.00

Comptes dépenses	Montant Budgété	Dépenses au 01.01.2015	Dépenses période rapport	Dépenses cumul au 31.05.2015	Solde comptable
316 Loyers, redevances	0.00	0.00	1,674.00	1,674.00	1,674.00-
363 Subv. accordées	0.00	0.00	5,619.50	5,619.50	5,619.50-
Total dépenses	0.00	0.00	7,293.50	7,293.50	7,293.50-

Comptes recettes	Montant Budgété	Recettes au 01.01.2015	Recettes période rapport	Recettes cumul au 31.05.2015	Solde comptable
463 Subv.Public ou Tiers	0.00	0.00	7,293.50	7,293.50	7,293.50
Total recettes	0.00	0.00	7,293.50	7,293.50	7,293.50

TOTAL	0.00			0.00	0.00

	Solde au 01.01.2015	Recettes période	Dépenses période	Nouveau solde
Calcul du solde	0.00	7,293.50	7,293.50	0.00

(budget initial non modifié)

Date	Signature(s) du(des) requérant(s)
16.06.2015	

Fonds..... : ME10061 SER/JST (ETHZ) 2014 SOLDATI-FAVRE DOMINIQUE
Financé par..... : UNIG Fonds institutionnel global
Période..... : 01.01.2015 au 31.05.2015
Centre financier : MIMOL
Requérant(s).... : SOLDATI-FAVRE DOMINIQUE

RUBRIQUE: 316 Loyers, redevances

Date comptabl	No pièce	Date pièce	Référence	Libellé	Compte	Contre partie	Ordre intern	Débit	Crédit	Solde
13.01.15	20000385	FI 13.01.15	KAMEYAMA KYOHKO	HOME SAINT-PIERRE PETERSHOFLI	3169000	F-108192		1,674.00		1,674.00- *
				SOLDE						1,674.00-
				TOTAL DE CETTE PERIODE				1,674.00	0.00	1,674.00-

Fonds..... : ME10061 SER/JST (ETHZ) 2014 SOLDATI-FAVRE DOMINIQUE
Financé par..... : UNIG Fonds institutionnel global
Période..... : 01.01.2015 au 31.05.2015
Centre financier : MIMOL
Requérant(s).... : SOLDATI-FAVRE DOMINIQUE

RUBRIQUE: 363 Subv. accordées

Date comptabl	No pièce	Date pièce	Référence	Libellé	Compte	Contre partie	Ordre intern	Débit	Crédit	Solde
14.01.15	134	FI 14.01.15		KAMEYAMA KYOKO/SER/JST EG 2014	3637003			3,572.50		3,572.50- *
03.03.15	1143	FI 03.03.15		KAMEYAMA KYOHKO	3637003			2,047.00		5,619.50- *
				SOLDE						5,619.50-
				TOTAL DE CETTE PERIODE				5,619.50	0.00	5,619.50-

Fonds..... : ME10061 SER/JST (ETHZ) 2014 SOLDATI-FAVRE DOMINIQUE
Financé par..... : UNIG Fonds institutionnel global
Période..... : 01.01.2015 au 31.05.2015
Centre financier : MIMOL
Requérant(s).... : SOLDATI-FAVRE DOMINIQUE

RUBRIQUE: 463 Subv.Public ou Tiers

Date comptabl	No pièce	Date pièce	Référence	Libellé	Compte	Contre partie	Ordre intern	Débit	Crédit	Solde	
13.01.15	100552	FI 17.12.14		ETHZ RG FELLOW 11.2014	4630991				7,293.50	7,293.50	*
				SOLDE						7,293.50	
				TOTAL DE CETTE PERIODE				0.00	7,293.50	7,293.50	

Fonds..... : ME10061 SER/JST (ETHZ) 2014 SOLDATI-FAVRE DOMINIQUE
Financé par..... : UNIG Fonds institutionnel global
Période..... : 01.01.2015 au 31.05.2015
Centre financier : MIMOL
Requérant(s).... : SOLDATI-FAVRE DOMINIQUE

Matric. Nom/prénom	Pér.	Salaire brut	Alloc. famil.	AVS AVS	AC AC	LAA LAA	APG APG	LPP LPP	Diverses Retenues	Autres sal	Sal. net Coût sal.

** = Charges employeur

Coût salarial (0.00) = Salaire brut (0.00) + Charges sociales (0.00)
Total employeur (0.00) = Coût salarial (0.00) + Provision 13ème brut (0.00) + Provision 13ème charges (0.00)