BASIC INFORMATION ON SUB-PROJECT

NAME OF PROGRAMME/FUND	Scholarship Fund - Sciex NMS^{ch}
RESEARCH FIELD AND OTHER RESEARCH FIELDS INVOLVED (if applicable)	Basic Biological Research, Chemistry
TITLE OF THE SUB-PROJECT	Evaluating the specific roles of apicomplexan aspartic proteases – prospective protocols to study hostvector-pathogen interactions (DS-APA)
REGION OF THE CZECH REPUBLIC (according to the location of the home institution)	Region of South Bohemia
GRANT AMOUNT SPENT	97 697,49 CHF
INTERMEDIATE BODY	Swissuniversities
HOME INSTITUTION	Academy of Sciences of the Czech Republic Biology Centre
HOST INSTITUTION	University of Geneva Facutly of Medicine
NAME OF THE FELLOW	Daniel Sojka

ABSTRACT OF THE SUB-PROJECT	Babesiosis is an emerging tick-borne illness that mimics malaria, puts more than half worlds cattle population is at risk and severe human cases frequently occur in immunocompromised populations. The causative agents Babesia sp. belongs to the large group of apicomplexan parasites and is related to malarial Plasmodium species that are intensively studied. The aim of this project is to také advantage of the "state of art" protocols to study endocytosis and proteolytic degradation within major apicomplexan models at host institution. Unraveling the molecular mechanisms implicated in transmission and virulence is required in in order to better understand the host-pathogen and hostvector interactions and to design novel specific drugs and vaccines for this disease. Based on the previous fellow's experience, we propose to explore the functions of aspartic proteases of group/clan C from Plasmodium (PfPMIX and PfPMX) and Toxoplasma (TgASP3). These proteases have already been characterized at the host institution. The objectives are to determined the specific function of each protease applying reverse genetic approaches in Toxoplasma gondii and Plasmodium berghei respectively. These genes have been extensively investigated in the Host laboratory and appear to be essential and conditional knock-down strategies will be applied. The localization and dynamics of these protease druing the lytic cycle of these parasites will be investigated by confocal microscopy. In parallel these enzymes will be recombinantly expressed, purified to define enzymatic properties, activation conditions and substrate specifity. Resolving the 3D structure by protein crystallization will be considered as a collaborative effort. The technologies will be than transfered to the Home laboratory and will be applied to Babesia research interests after returning to the home institution.

MAIN RESULTS	***· Using gene targeting recombinant technique (knock-in), we have constructed a T. gondii line expressing C-terminal -Ty epitope tag from the endogenous TgASP5 locus. The cloned line was verified both by PCR with specific primers and by Western blot using anti-Ty monoclonal antibodies.*** ***· Unlike plasmepsin V located in the endoplasmatic reticulum, TgASP5 localizes specifically to Golgi as shown by IF labeling of the knocked-in C terminal-Ty TgASP5 strain co-expressing T. gondii Golgi marker-YFP fusion protein (Golgi reassembly and stacking protein, GRASP; yellow fluorescent protein, YFP)*** ***· We have also used the TgASP5 gene knock-in strategy to interrupt the reading frame of the endogenous TgASP5 locus with a truncated N terminal part of TgASP5 (excluding the active peptidase sites) and C terminally tagged with Ty (Knock-in/Knock-out). Transformed parasites showed almost no ability to survive under the selection and no signal has been detected from repeated IFAs. This indicated an essential role of TgASP5.*** ***· Since RNAi approach seems to be in its infants and could not be trustfully used to evaluate T. gondii gene/protein functions5, for the haploid asexual parasite stages, if a gene of interest fulfills a critical role for survival, a conditional gene knock-down is an optimal strategy to achieve transgenic lineages. These mainly include expression of the gene of interest from a tetracycline operon controlled promoter, introduction of a destabilization domain (DD) or utilizing the Cre-lox site-specific recombinase technology system by an introduction of two independent Lox P sites in the locus of interest of a Cre recombinase expressing parasite cell line. We have designed 5 different approaches based on the above given options for TgASP5. The plasmids have been mostly constructed and the parasites are currently being transfected and selected for positive clones. The (Tet)-based transcriptional regulation of TgASP5 second copy under a stable expressing promoter was shown to work efficiently and thus remains our priorized approach to achieve TgASP5 conditional knock-downs.*** ***· The T. gondii parasite clone expressing TgASP5 with the C terminal Ty epitope tag is currently used for metabolic labeling techniques. The overall goal is to obtain the immuno-precipitated native enzyme employing the anti-TY monoclonal antibodies. The pulled down TgASP5 should be tested for activity by affinity labeling with the activity-based probe FAP-09 in the presence/absence of the cathepsin D inhibitor pepstatin A and by assays with fluorescently labeled peptidyl substrates such as Abz- KPAEFFRL.*** ***· In parallel, a recombinatly expressed 6x histidine tagged E. coli TgASP5 fragment (excluding the C-terminal transmembrane domain) is being prepared and will be used for obtaining TgASP5 polyclonal rabbit antibodies and tested for activity with cathepsin-D specific activity-based probe FAP-09 and Abz-KPAEFFRL substrate.*** ***tested for activity with cathepsin-D specific activity-based probe FAP-09 and Abz-KPAEFFRL substrate.*** ***Publikace:*** Hammoudi P.M., Jacot D., Mueller C., Di Cristina M., Dogga S.K., Marq J.B., Romano J., Tosetti N., Dubrot J., Emre Y., Lunghi M., Coppens I., Yamamoto M., Sojka D., Pino P., Soldati-Favre D. (2015) Fundamental Roles of the Golgi-Associated Toxoplasma Aspartyl Protease, ASP5, at the Host-Parasite Interface *PLoS Pathogens* **11**: e1005211.

DATE OF REALISATION OF THE FELLOWSHIP	1.10.2012 - 30.9.2013
MORE INFORMATION ON THE PROGRAMME	**www.sciex.ch**