Samuel Pessoa Conference – SPC001

Growth control in Trypanosomes: What is new after the genome?

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Protozoan parasites such as *Trypanosoma* and *Leishmania* species have evolved by adapting to different host environments. It is expected that the best fittings have been selected based on a balanced propagation of the parasite in both insect and vertebrate hosts. Therefore, these organisms appearing early in evolution may have developed mechanisms to sense changes in the ambient. Remarkably, trypanosomes do not control gene expression by changing the levels of transcription of specific messages. Rather, they use a general control of transcription regulating gene expression by increasing or decreasing overall DNA transcription. Specific gene expression is then regulated by selecting mRNAs for processing and translation, or by increasing the total gene copy number. In this talk, I intend to summarize these regulatory mechanisms facing new data available from genomic, transcriptome and proteomic databases. It is remarkable that trypanosomes display a large collection of protein kinases, phosphatases, acetylases, deacetylases and other protein modifying enzymes involved in the signaling responses, when compared to other organisms. These enzymes present poorly conserved domains, indicating unique signaling cascades. Therefore, understanding the mechanisms involved in growth control is a new challenge in trypanosomatid biology.

In my presentation, I'll describe our studies focusing in two major questions related to growth control. One is whether chromatin modifications affect transcription control and how environmental stimuli promote these modifications. Our group found that both proliferating forms of *Trypanosoma cruzi* present high levels of transcription and that stresses provided by genotoxic agents, high temperatures and starvation induces transcriptional arrest. Our data suggest that phosphorylation/dephosphorylation of the largest subunit of RNA polymerase II is a key regulatory event related to transcription elongation, while induction of deacetylation of histones appear to cause inhibition of transcription initiation.

The other question that will be addressed is how the environment affects protein translation. In eukaryotes, translation control is mainly regulated at the initiation level. Our group demonstrated that phosphorylation of the α-subunit of the eukaryotic initiation factor 2 (eIF2α), a key factor in the translation control, is involved in the differentiation of epimastigotes in metacyclic trypomastigotes. Our results indicate that parasite differentiation require protein synthesis arrest following starvation. When eIF2α phosphorylation is inhibited, no full protein synthesis arrest occurs resulting in poor differentiation. I'll also show variations of cellular localization of protein kinases involved in eIF2α phosphorylation according to the parasite growth phase, which would explain how the differentiation is controlled. In another set of experiments, I'll provide evidences that phosphorylation of eIF5A, , is also required for protein synthesis and its dephosphorylation under stress conditions prevents cell damage. eIF5A is a protein with an elusive function in translation, and our data provide the first evidence that it may act at the level of the first peptide bond formation. Finally, we will show results illustrating how protein kinases known as target of rapamycin (TOR) kinases respond to environmental stresses, and what are the effectors involved their activation in trypanosomes.

All these results highlight some of the mechanisms used by trypanosomes to sense and adapt each situation during their life cycles. Nonetheless, there are still more questions to be answered in the near future. For example, is unknown what are the proteins involved in the signaling cascades, and very few of them have been identified in the genome. Probably, they have unusual characteristics when compared to other eukaryotes. These open new perspectives not only to understand the biology of trypanosomes, but

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Closure Conference – CC001

Roles of the Kallikrein-Kinin System in the Pathogenesis of Chagas (Heart) Disease: New Lessons from Experimental Models

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Nearly a century after the discovery of Chagas disease, we have come to realize that it is still difficult to predict the clinical outcome of heart disease in chronically infected patients. Among other obstacles, we lack appropriate infection models to investigate the molecular determinants of the phenotypic variability of the genetically diversified *Trypanosoma cruzi* species. Although limited to a single *T. cruzi* strain (Dm28c; DTU I), progress in the analysis of the proteolytic mechanisms influencing host/parasite balance in the perivascular environment (revised by Scharfstein and Andrade, 2011) may stimulate new initiatives to address this challenging problem experimentally. Using intravital microscopy in combination with infection models established with transgenic mice, our group has demonstrated that tissue culture trypomastigotes (TCTs) elicit inflammatory edema and invade cardiovascular cells through the activation of kallikrein-kinin system (KKS), a hub-like proteolytic network that couples the "contact" phase coagulation pathway to immunity via generation of proinflammatory peptides, such as bradykinin and complement anaphylatoxins. As explained further below, the generation of vasoactive "kinins" in extravascular sites of *T. cruzi* infection depends on the molecular interplay between two well-characterized *T. cruzi* molecules: (i) tGPI, a lipid anchor that was previously characterized by Almeida and Gazzinelli (2001) as a developmentally regulated TLR2 ligand of trypomastigotes, and (ii) cruzipain, a lysosomal cysteine protease that liberates the decapeptide LBK from an internal moiety of high-molecular weight kininogen (HK) (reviewed by Scharfstein and Andrade, 2011). Acting synergistically, tGPI and cruzipain trigger microvascular responses that are propagated at expense of the proteolytic activation of mast cell/KKS pathway. Before outlining the functional consequences of KKS activation in peripheral sites of infection, it may be helpful to briefly describe the activation pathways involved in the initiation/expansion of the inflammatory response triggered by Dm28c TCTs. At the onset of infection, innate sentinel cells (e.g., tissue macrophages) sense the presence of tGPI shed by trypomastigotes via TLR2. Next, the activated macrophages secrete TNF-α and CXC chemokines (KC/MIP-2), which in turn increase vascular permeability through CXCR2-dependent activation of endothelium/neutrophils (Schmitz *et al.*, 2009). Leading to the accumulation of blood-borne plasma proteins (including HK) in the parasite-laden extravascular tissues, the neutrophil-evoked transudation is the rate-limiting step (TLR2/CXCR2-dependent) controlling the cruzipain-driven liberation of kinins, further downstream in the progression of the inflammatory cascade. Acting as a paracrine hormone, the short-lived LBK activates endothelium bradykinin B2 receptors (BK2R). Feedback cycles of plasma leakage, kinin release and BK2R signaling further intensify the interstitial edema in sites of infection.

Several years ago, we hypothesized that the liberation of kinins in inflamed peripheral tissues may play antagonist roles in the host/parasite balance. The groundwork behind this proposition came from in vitro studies showing that kinins proteolytically released by Dm28c TCTs potentiate the invasion of non-phagocytic host cells (e.g., cardiomyocytes, smooth muscle and endothelium cells) through the signaling of BK2R (Scharfstein et al., 2000). Although not mutually exclusive, we subsequently showed that Dm28c TCTs may opportunistically invade cardiovascular cells through an inducible "gate of entry", i.e., BK1R, a subtype of BKR whose expression is strongly upregulated in injured/inflamed tissues (reviewed by Scharfstein and Andrade, 2011).

Although antigen-driven immunopathology is considered the primary mechanism leading to chronic myocarditis, experts from the vascular field have long claimed that tissue hypoxia, presumably a sequel of infection-associated microvasculopathy (Rossi *et al*., 1990; Morris *et al*., 1990; Higuchi *et al*., 1999) might aggravate collateral tissue damage inflicted by pathogenic subsets of effector T cells (Silverio *et al*., 2012). Focusing on the endothelin pathway, Tanowitz et al. (1999) obtained evidences that the altered vascular tone induced by *Trypanosoma cruzi* is linked to the vasoconstrictor function of endothelins. After reporting that parasitized cardiomyocytes upregulate the expression of endothelin, Petkova *et al*.(2000) suggested that the aberrant response of infected cardiomyocytes could contribute to cardiac remodeling in

Chagas disease. In an elegant study involving chronically infected mice, these authors observed that heart fibrosis was significantly attenuated in transgenic animals in which the endothelin gene was specifically removed from cardiomyocytes, while ablation this gene in endothelial cells did not significantly reduced cardiac remodeling (Tanowitz *et al*., 2005). While these studies were in progress, our studies of the proinflammatory phenotype of Dm28c TCTs revealed a functional link between the endothelin and kinin signaling pathways (Andrade et al., 2012; reviewed by Scharfstein and Andrade, 2011). In view of the precedent that kinins/BK2R drive the endocytic uptake of Dm28c TCTs via the $[Ca^{2+}]_i$-dependent pathway (Scharfstein et al., 2000), we hypothesized that the parasites may take advantage of the availability of kinins and endothelins in the inflamed heart to invade cardiovascular cells more efficiently through the activation of ETRs/BK2R. Indeed, using subtype specific ETR blockers or iRNA in invasion assays carried out with primary human smooth muscle cells, we obtained evidences that parasite infectivity involves, at least in part, cooperative activation between ETaR, ETbR and BK2R (Andrade et al., 2012). Interestingly, we observed that parasite infectivity was not further reduced by the combined addition of ETR and BKR blockers to smooth muscle cells, thus implying that BK2R/ETRs are interdependently activated by TCT Dm28cs. After observing that ETRs were clustered at sites of parasite attachment, Andrade et al. (2012) checked whether cholesterol-depleting drugs (reconstituted or not with exogenous cholesterol) could abolish parasite uptake via ETRs/BK2R. The results confirmed that this was the case, implying that ETRs/BKRs might act as a single-functional (signaling) unit, most likely embedded inlipid rafts. Future studies may clarify whether BKRs/ETRs integrate the multimolecular signaling platforms associated to ceramide rafts. This hypothesis merits investigation in light of recent evidences that trypomastigotes invade non-phagocytic cells by subverting the acid sphingomyelinase/ceramide-dependent mechanism of repair from plasma membrane injury (Fernandes et al., 2011).

Traditionally referred as the “contact-phase” (intrinsic) pathway of coagulation, the KKS is composed of 3 serine protease zymogens [FXII (Hageman Factor), Plasma Kallikrein (PK) and FXI) and HK, a multifunctional adaptor protein, which, as previously mentioned, is a BK precursor molecule. Similar to the effects induced by silica, pathogens displaying negatively charged surface molecules are able to activate the contact phase coagulation system. In addition, it was recently demonstrated that the KKS is also endogenously activated by platelet-derived polyphosphates and mast-cell derived heparin (Oschatz et al., 2011). Irrespective of the origin of the contact phase activator, the KKS cascade is initiated by reciprocal cleavage between FXIIa/PKa. After several feedback cycles of activation, PKa releases vasoactive BK from HK. Focusing on immunity, Monteiro et al. (2006) reported that conditions leading to the full-fledge activation of the KKS in *T. cruzi*-infected mice lead to the activation of BK2R expressed by immature $CD11c^+$ dendritic cells (DCs) (Monteiro et al., 2007). After migrating to the T cell-rich areas of secondary lymphoid tissues, the IL-12-producing DCs guide TH1 effector development.

Awareness that mast cells are central players in cardiac remodeling, including in Chagas’ disease (Meuser-Batista et al., 2011) led us to investigate whether Dm28c may evoke an intracardiac edema via the mast cell/KKS pathway. Strategically localized in the perivascular region, these innate sentinel cells may integrate the trans-cellular cross-talk forged by the TLR2/CXCR2/ETR/BKR axis because (i) they express the innate receptor TLR2 (Carlos et al., 2009) and (ii) present endothelins in their storage granules (Ehrenreich *et al*., 1992). Using the hamster cheek pouch model of Dm28c infection, Nascimento and co-workers (see accompanying abstract) showed evidence that bradykinin-induced microvascular leakage is strongly enhanced by low levels of histamine, a mast cell mediator. More recently, we developed a model of intracardiac infection to evaluate whether Dm28c TCTs induce interstitial edema via the mast cell/KKS pathway. Using the intracardiac infection model, we sought to determine whether the parasites could take advantage of the transient availability of kinins and endothelins (“infection-promoting GPCR ligands”) in the transiently inflamed heart tissues to invade cardiovascular cells via BK2Rs/BK1R/ETaR/ETbR. Guided by high-resolution echocardiography (see accompanying abstract by Andrade et al.), Dm28c TCTs were injected in the left ventricle of C57BL/6 $BK2R^{+/+}$(wt), $BK2R^{-/-}$ or Balb/c (naïve). One hour before the intracardiac inoculation of parasites, wt mice were treated with a single dose (systemic) of subtype specific GPCR antagonists. Analysis of pathogenic outcome included assessment (confocal microscopy) of intracardiac oedema (2 h p.i.); parasite load and mRNA levels of chemokines/cytokine in heart tissues (qPCR;3 d p.i); myocarditis and fibrosis at (30 d p.i.).

Consistent with our working hypothesis, we found that Dm28c TCTs evoked interstitial edema in cardiac tissues of $BK2R^{+/+}$ mice, but not in $BK2R^{-/-}$ mice. Furthermore, injection of a single-dose of BK2R, BK1R or ETRaR/ETbR antagonists blocked the intracardiac edema of wild-type infected mice. Strikingly, although these GPCR antagonists were administered in single (systemic) dose, shortly before intracardiac injection of the pathogens, this punctual intervention had long lasting influence on pathogenic outcome. For example, the intracardiac parasite load (measured 3 days p.i.) was markedly reduced by these GPCRs or by drugs that interfered with the mast cell/KKS pathway. Importantly, histopathological examinations made 30 d p.i. confirmed that that the BKR or ETR antagonists significantly reduced infection-associated myocarditis and fibrosis.

Despite the limitations of this artificial model, our studies suggest that intramyocardial edema, a sequel of Dm28c trypomastigote-elicited activation of the mast cell/KKS pathway, might influence host/parasite equilibrium and the pathologic outcome of *T. cruzi* infection in heart tissues. From a practical viewpoint, the finding that Dm28 trypomastigotes rely on cooperative activities of tGPI and cruzipain to activate the mast cell/KKS-dependent edematogenic pathway of inflammation may serve as a paradigm for the investigation of the molecular determinants of phenotypic variability in natural populations of *T. cruzi*.

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Selected Bibliography:

Almeida IC, Gazzinelli RT (2001). Proinflammatory activity of glycosylphosphatidylinositol anchors derived from *Trypanosoma cruzi*: structural and functional analyses. J Leukoc Biol 70: 467-477.

Andrade D, Serra R, Svensjö E, Lima AP, Ramos ES Jr, Fortes FS, Morandini AC, Morandi V, Soeiro Mde N, Tanowitz HB, Scharfstein J. (2012). Trypanosoma cruzi invades host cells through the activation of endothelin and bradykinin receptors: a converging pathway leading to chagasic vasculopathy. Br J Pharmacol. 165:1333-47.

Carlos D, Frantz FG, Souza-Júnior DA, Jamur MC, Oliver C, Ramos SG, Quesniaux VF, Ryffel B, Silva CL, Bozza MT, Faccioli LH (2009). TLR2- dependent mast cell activation contributes to the control of Mycobacterium tuberculosis infection. Microbes Infect 11(8-9):770-778.

Ehrenreich H, Burd PR, Rottem M, Hültner L, Hylton JB, Garfield M, Coligan JE, Metcalfe DD, Fauci AS (1992). Endothelins belong to the assortment of mast cell-derived and mast cell-bound cytokines. New Biol 4(2):147-156.

Fernandes MC, Cortez M, Flannery AR, Tam C, Mortara RA, Andrews NW. (2011). Trypanosoma cruzi subverts the sphingomyelinase-mediated plasma membrane repair pathway for cell invasion. J Exp Med. 208(5):909-21.

Higuchi ML, Fukasawa S, De Brito T, Parzianello LC, Bellotti G, Ramires JA (1999). Different microcirculatory and intersticial matrix patterns in idiopathic dilated cardiomyopathy and Chagas' disease: a three dimensional confocal microscopy study. Heart 82: 279-285.

Lima AP, Almeida IC, Tersariol ILS, Schmitz V, Schmaier AH, Juliano L, Hirata IY, Müller-Esterl W, Chagas JR, Scharfstein J (2002). Heparan sulfate modulates kinin release by *Trypanosoma cruzi* through the activity of cruzipain. J Biol Chem 277(8): 5875-5881.

Meuser-Batista M, Corrêa JR, Carvalho VF, de Carvalho Britto CF, Moreira OC, Batista MM, Soares MJ, Filho FA, E Silva PM, Lannes-Vieira J, Silva RC, Henriques-Pons A. (2011). Mast cell function and death in Trypanosoma cruzi infection. Am J Pathol. 179(4):1894-904.

Monteiro AC, Schmitz V, Svensjö E, Gazzinelli RT, Almeida IC, Todorov A, de Arruda LB, Torrecilhas AC, Pesquero JB, Morrot A, Bouskela E, Bonomo A, Lima AP, Müller-Esterl W, Scharfstein J (2006). Cooperative activation of TLR2 and bradykinin B2 receptor is required for induction of type 1 immunity in a mouse model of subcutaneous infection by *Trypanosoma cruzi.* J Immunol 1;177(9):6325-35.

Monteiro AC, Schmitz V, Morrot A, de Arruda LB, Nagajyothi F, Granato A, Pesquero JB, Müller-Esterl W, Tanowitz HB, Scharfstein J (2007). Bradykinin B2 Receptors of dendritic cells, acting as sensors of kinins proteolytically released by *Trypanosoma cruzi*, are critical for the development of protective type-1 responses. PLoS Pathog 3(11):e185.

Oschatz C, Maas C, Lecher B, Jansen T, Björkqvist J, Tradler T, Sedlmeier R, Burfeind P, Cichon S, Hammerschmidt S, Müller-Esterl W, Wuillemin WA, Nilsson G, Renné T.(2001). Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo. Immunity.34(2):258-68

Petkova SB, Tanowitz HB, Magazine HI, Factor SM, Chan J, Pestell RG, Bouzahzah B, Douglas SA, Shtutin V, Morris SA, Tsang E, Weiss LM, Christ GJ, Wittner M, Huang H (2000). Myocardial expression of endothelin-1 in murine *Trypanosoma cruzi* infection. Cardiovasc Pathol 9 (5):257-265.

Scharfstein J, Schmitz V, Morandi V, Capella MM, Lima AP, Morrot A, Juliano L, Müller-Esterl W (2000). Host cell invasion by *Trypanosoma cruzi* is potentiated by activation of bradykinin B2 receptors. J Exp Med 192:1289-1300.

Scharfstein J, Andrade D. (2011) Infection-associated vasculopathy in experimental Chagas disease pathogenic roles of endothelin and kinin pathways. Adv Parasitol. 76:101-27.

Scharfstein J, Andrade DS (2011). Bradykinin, Endothelin and Vasculopathy. In: Advances in Parasitology. Chagas Disease: *Trypanosoma cruzi,* the first hundred years. Academic Press: Elsevier Limited, In press.

Schmitz V, Svensjö E, Serra RR, Teixeira MM, Scharfstein J (2009). Proteolytic generation of kinins in tissues infected by *Trypanosoma cruzi* depends on CXC chemokine secretion by macrophages activated via Toll-like 2 receptors. J Leukoc Biol 85 (6):1005-1014.

Silverio JC, Pereira IR, Cipitelli Mda C, Vinagre NF, Rodrigues MM, Gazzinelli RT, Lannes-Vieira J. (2012). CD8+ T-cells expressing interferon gamma or perforin play antagonistic roles in heart injury in experimental Trypanosoma cruzi-elicited cardiomyopathy. PLoS Pathog. 8(4):e1002645.

Tanowitz HB, Wittner M, Morris SA, Zhao W, Weiss LM, Hatcher SA, Braunstein VL, Huang H, Douglas SA, Valcic M, Spektor M, Christ GJ (1999). The putative mechanistic basis for modulatory role of endothelin-1 in the altered vascular tone induced by *Trypanosoma cruzi.* Endothelium 6:217-230.

Tanowitz HB, Huang H, Jelicks LA, Chandra M, Loredo ML, Weiss LM, Factor SM, Shtutin V, Mukherjee S, Kitsis RN, Christ GJ, Wittner M, Shirani J, Kisanuki YY, Yanagisawa M (2005). Role of endothelin 1 in the pathogenesis of chronic chagasic heart disease. Infect Immun 73 (4):2496-2503.

CO001 - The Control and Pathology in Leishmania braziliensis

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In area of L.braziliensis transmission 70% of the individuals exposed to L. braziliensis infection do not develop disease while cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML) are characterized by high production of pro-inflammatory cytokines and development of pathology sub-clinical L. braziliensis infection (SC) is characterized by a positive delayed type hypersensitivity test (DTH) and production of low amount of IFN-γ and TNF-α. Here we evaluate the mechanism involved in the weak type 1 immune response of individuals with SC L. braziliensis and how they control leishmania infection. Initially we determine if the low type 1 immune response in these subjects was due to a modulation of the immune response mediated by cytokines and T reg cells. To determine the ability to control leishmania infection we compare the production of cytokines by macrophages and the ability of these cells to kill leishmania from individuals with different clinical forms of L.braziliensis infection. Cytokines and chemokines were measured in lymphocyte and macrophage cultures by ELISA and PCR. T reg cells were analyzed by FACS analysis and function. The production of IL-10 and IL-27 were not enhanced in SC and neutralization of IL-10 did not enhance IFN-γ production in these individuals. While macrophages from CL and ML patients produced higher amounts of CXCL9, CXCL10 and TNF-α than SC subjects, killing of L. braziliensis was higher in SC individuals than in CL and ML patients. These data show that macrophages and lymphocytes from CL and ML patients produce higher levels of pro-inflammatory chemokines and cytokines that were associated with pathology than SC macrophages. In contrast, macrophages from SC individuals kill more efficiently L. braziliensis than macrophages from CL and ML, indicating that protection is associated with the innate immune response.

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CO002 - Epidemiology and control of frontier malaria in Brazil

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Malaria is amongst the major infectious diseases for which several decades of intensive control efforts have met only partial success in Brazil. Nearly 300,000 malaria cases are still reported each year in Brazil, 99.9% of them in the Amazon Basin. Malaria transmission typically clusters in mining and logging camps and farming settlements, which not only induce massive environmental changes (such as deforestation) that alter vector biology and favor malaria transmission, but also attract non-immune migrants to areas full of natural vector breeding sites. Malaria control currently focuses on early diagnosis and treatment of clinical cases to reduce transmission and morbidity; a large network of malaria diagnosis outposts provides free microscopy-based diagnosis and free treatment of laboratory-confirmed infections. Vector control measures, particularly cyclic house spraying with insecticides, were gradually phased out over the past decade and little locally generated research has addressed alternative tools or interventions that could improve current control strategies. The paucity of scientific evidence to support current malaria-control interventions in Brazil is particularly surprising for a middle-income country with relatively well-developed research capacity. This situation calls for an enhanced partnership between researchers and decision-makers, to face the country's challenges in this area.

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CO003 - From Genomes to Host-Pathogen Infectomes: Models in profiling host-pathogen interactions

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We have adopted a novel approach aimed at characterizing host-pathogen infectomes. We define the infectome as the component of the pathogen's genome/transcriptome/proteome that allows it to subvert the functions of host cell molecular machineries, receptors, and signaling proteins, as well as the portion of the host cell's -omes that play a role in the infection process. Our screens include the use of a combination of 1) bioinformatic tools aimed at predicting surface and secreted components, 2) simultaneous interrogation of the host and pathogen transcriptomes during infection and intracellular survival and 3) high-throughput protein-protein interaction screens between a selection of host and pathogen proteins informed by the first two steps. The application of this approach to Trypanosoma cruzi and Leishmania major, two intracellular pathogens that parasitize mammalian cells is yielding significant biological insights into host-pathogen interactions. I will specifically discuss the investigation of T. cruzi Mucin-Associated Surface Proteins (MASP), their human protein interaction partner and their apparent role in host cell invasion.

MASPs are members of a multigenic family recently identified during the sequencing of the T. cruzi CL Brener genome. This family contains around 1,400 members, consisting of approximately 6% of the diploid genome. Highly conserved N- and C-terminal domains, which encode a signal peptide and GPI-anchor addition site respectively, and a hypervariable central region, characterize MASPs. Members of this family are predominantly expressed in the infective trypomastigote form. We hypothesized that members of the T. cruzi MASP protein family play a major role in the interaction of the parasite with the host cell. In order to investigate a putative role for T. cruzi MASP at the host-pathogen interface, we used MASP as a bait protein against the human proteome using a high-throughput platform that we have recently established for identifying protein-protein interactions between pathogens and theirs hosts. Yeast two-hybrid screens identified human SNAPIN as one of two major MASP interacting proteins. SNAPIN is a member of the SNARE protein complex, which may have a role in a calcium-dependent exocytosis. The MASP-SNAPIN interaction was further validated using in vivo co-Affinity Purification and in vitro pull-down assays. Immunofluorescence assays showed human SNAPIN is recruited to the parasite surface during invasion. Co-localization experiments indicated that SNAPIN is associated with the late endosomes and lysosomes. Supporting our initial hypothesis, SNAPIN depletion using siRNA oligomers in HeLa cells and snapin-/- in Mouse Embryonic Fibroblast (MEF) cells significantly inhibited T. cruzi invasion, suggesting a role for SNAPIN in this process. Lysosomes in snapin-/- MEF cells displayed aberrant morphology and distribution and the parasites did not recruit host lysosomes efficiently when compared to wild-type cells. This was likely due to an impaired calcium-dependent lysosome exocytosis in snapin-/- MEF cells. SNAPIN was translocated to the plasma membrane upon calcium influx induced by a calcium ionophore (Ionomycin), resulting in the exposure of the luminal domain of SNAPIN to the extracelluar space. Leishmania tarentolae transgenic strains expressing two different MASP proteins were shown to trigger intracellular calcium transients in HeLa cells, presumably by injuring the cell membrane. We propose that T. cruzi MASP plays a role in wounding the plasma membrane of the host cell, which in turn elicits a transient intracellular calcium flux and leads to the translocation of lysosome-associated SNAPIN to the plasma membrane. Human SNAPIN, through its exposed luminal domain would then provide an anchor for the entry to the parasite into the cell. The mechanism of T. cruzi MASP evoked calcium influx in the host cell membrane remains under investigation.

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CO004 - Base J, biosynthesis and function of a minor base in the nuclear DNA of kinetoplastida

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In 1978, George Cross and I set out to unravel the mechanism of antigenic variation in African trypanosomes. George had shown that the surface coat of T. brucei consists of a single protein, the Variant Surface Glycoprotein (VSG). Soon we found that VSG genes are expressed from a telomeric expression site (ES) and that T. brucei switches coat by replacing the VSG gene in the ES by a different one (1). Unexpectedly, we subsequently found that there are multiple ESs and that T. brucei can also switch coat by switching ES. In the process of switching off an ES, some restriction sites in the ES became blocked and after a long search we found that this is due to replacement of DNA base T by base J (glucosyl-hydroxy-methyluracil). In Leishmania about 1% of T is replaced by J and 99% of J is in telomeric repeats. Biosynthesis of J is initiated by 2 thymine hydroxylases, J-binding protein (JBP) 1 and 2 (2). The function of base J was long elusive, but we recently found that the positions where RNA polymerase II stops transcription are also marked by J, called internal J (iJ). Induced loss of iJ leads to massive transcriptional readthrough and death of the parasite, making J biosynthesis an interesting new drug target in Leishmania (3).

Reference List

(1) Borst P, Cross GAM. Molecular basis for trypanosome antigenic variation. [Review]. Cell 1982;29:291-303.

(2) Borst P, Sabatini R. Base J: discovery, biosynthesis and possible functions. Ann Rev Microbiol 2008;62:235-51.

(3) van Luenen H, Farris C, Jan S, Genest P-A, Tripathi P, Velds A, et al. Glucosylated hydroxymethyluracil (DNA base J) prevents transcriptional read-through in Leishmania. Cell. In press, 2012.