

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also 32: 14803]

14842. **Cordon-Obras, C., Berzosa, P., Ndong-Mabale, N., Bobuakasi, L., Buatiche, J. N., Ndongo-Asumu, P., Benito, A. & Cano, J., 2009.** *Trypanosoma brucei gambiense* in domestic livestock of Kogo and Mbini foci (Equatorial Guinea). *Tropical Medicine and International Health*. **e Publication ahead of print March 23.**

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To evaluate *Trypanosoma brucei gambiense* infection in peri-domestic livestock from Kogo and Mbini foci (Equatorial Guinea) in order to investigate its possible implication in the sleeping sickness transmission cycle in these hypoendemic foci, samples from 698 domestic animals (goats, sheep and pigs) from trypanosomiasis-endemic localities of Kogo and Mbini foci were tested for animal trypanosomes and *T. b. gambiense* (group I) by species-specific polymerase chain reaction. *Trypanosoma brucei s.l.*, the predominant trypanosome species, was detected in 182 (52.6 percent) samples from Mbini and in 127 (36.1 percent) samples from Kogo. *T. b. gambiense* was only identified in seven (2 percent) of the Mbini samples and one co-infection (with *T. vivax*) was observed. The occurrence of *T. b. gambiense* in peri-domestic livestock in Mbini and its absence in Kogo could explain the epidemiological differences between the two foci and could have significant implications for sleeping sickness control in Equatorial Guinea.

14843. **Enwezor, F. N., Umoh, J. U., Esievo, K. A., Halid, I., Zaria, L. T. & Anere, J. I., 2009.** Survey of bovine trypanosomosis in the Kachia grazing reserve, Kaduna State, Nigeria. *Veterinary Parasitology*, **159** (2): 121-125.

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This study assessed the prevalence of trypanosomes in cattle at the Kachia grazing reserve (KGR) in March and June 2004 and in February 2005. A total of 1 293 cattle blood samples were collected at random. The samples were analysed using the buffy coat technique and Giemsa thin blood films for parasite detection and identification. The effects of herd pen location to watering and grazing point's distances (using the global positioning system (GPS)) were determined and the mean packed cell volume (PCV) assessed. Overall, the detected prevalence of trypanosomosis was 8.4 percent, much higher than the previous prevalence of 5.3 percent before the present study was conducted. The prevalences in the months of March, June (2004) and February (2005) were 2.3 percent, 11.6 percent and 15.4 percent, respectively. Increased prevalence was associated with proximity of herd pens to watering point's distances (χ^2 for linear trend=4.447, $P<0.05$), but no association of herd pens to

grazing point distances ($\chi^2=2.186$, $P>0.05$); suggesting that hydrological network played an important part in trypanosomiasis transmission. The mean PCV of parasitaemic and aparasitaemic cattle were respectively 25.99 \pm 1.82 percent and 29.31 \pm 1.70 percent. The drop in mean PCV was most in 0-1-year age group, 23.47 \pm 3.10 percent and was statistically significant ($P<0.05$), suggesting that anaemia was most pronounced in this age group. Factors that may have contributed to the increased prevalence obtained were collapse of control measures and breed susceptibility. Since Zebu cattle were the predominant breeds in the reserve, the study advocates effective use of insecticide impregnated screens (traps and targets) with community participation in mind for sustainability. If government intervenes through PATTEC, ground spraying of insecticides in the reserve is recommended. In addition, chemotherapy and chemoprophylaxis should be systematically used to fight the problem of trypanosomiasis in the KGR towards improved livestock production.

14844. **Ezeani, M. C., Okoro, H., Anosa, V. O., Onyenekwe, C. C., Meludu, S. C., Dioka, C. E. & Azikiwe, C. C., 2008.** Immunodiagnosis of bovine trypanosomiasis in Anambra and Imo states, Nigeria, using enzyme-linked immunosorbent assay: zoonotic implications to human health. *Journal of Vector Borne Diseases*, **45** (4): 292-300.

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The prevalence of trypanosomiasis was studied in cattle, being a major source of animal protein in Nigeria and thus a very likely means of spread of human African trypanosomiasis (HAT). Enzyme-linked immunosorbent assay (ELISA) was used to diagnose bovine trypanosomiasis in 264 samples collected from adult cattle of mixed breeds, age and sex, in Anambra and Imo States, Nigeria. Out of 264 samples analysed, 21 (7.96 percent) were seropositive for *Trypanosoma congolense* while 20 (7.58 percent) were seropositive for *T. vivax* and 8 (3.03 percent) were seropositive for *T. brucei* infections in both the states. Thus, the predominant species was found to be *T. congolense*. Mixed infection of three species, *T. vivax*, *T. congolense* and *T. brucei* were found to dominate other mixed infections in both the states. ELISA detected the infection of the three species of trypanosomes in the same group of animals. The usefulness of antigen capture ELISA in the diagnosis of human or animal trypanosomiasis was established, and the possibility of the spread of HAT caused by *T. brucei gambiense* and *T. b. rhodesiense* through cattle was expressed.

14845. **Mamabolo, M. V., Ntantiso, L., Latif, A. & Majiwa, P. A., 2009.** Natural infection of cattle and tsetse flies in South Africa with two genotypic groups of *Trypanosoma congolense*. *Parasitology*, **136** (4): 425-431.

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The polymerase chain reaction was used to detect trypanosomes in samples collected from cattle, wild animals and tsetse flies in KwaZulu-Natal Province, South Africa. A total of 673 samples from cattle and 266 from tsetse flies in the study area located near the Hluhluwe-Umfolozi game reserve were analysed. Both *Trypanosoma congolense* and *T. vivax* were found as single or mixed infections in cattle and tsetse flies. Moreover, the *T. congolense* in

the infections were found to comprise two genotypic groups: the Savannah-type and the Kilifi-type, which were present either as single or mixed infections in cattle and in tsetse flies.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 32: 114805]

14846. **Berlin, D., Loeb, E. & Baneth, G., 2009.** Disseminated central nervous system disease caused by *Trypanosoma evansi* in a horse. *Veterinary Parasitology*, **161** (3-4): 316-319.

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Trypanosomiasis caused by *Trypanosoma evansi* ("Surra") is mainly a wasting disease affecting equids, camels and cattle as well as other domestic and wild animal species. In horses, infection may cause severe neurological abnormalities; however, the clinical progression, pathogenesis and molecular ante-mortem detection of this form of the disease have not been described in detail. A mare with progressive ataxia, head tilt, nystagmus and cranial nerve deficits submitted to treatment was diagnosed with central nervous system trypanosomiasis following the detection of a *Trypanosoma* trypomastigote in cerebrospinal fluid cytology. Histopathology following necropsy showed that the brain, spinal cord and kidneys were the main affected tissues with disseminated multifocal non-suppurative meningoencephalitis of the central nervous system and membranoproliferative glomerulonephritis. Serology for *T. evansi* was positive and PCR indicated the presence of parasite DNA in the cerebellum, brain stem, spinal cord and bone marrow but not in other organs and confirmed the identity of causative agent as *T. evansi*. This is the first report of ante-mortem detection of *T. evansi* in the cerebrospinal fluid of a horse and the first description of post-mortem PCR identification of the parasite DNA in the nervous system.

14847. **Mbaya, A. W., Aliyu, M. M. & Ibrahim, U. I., 2009.** The clinico-pathology and mechanisms of trypanosomosis in captive and free-living wild animals: a review. *Veterinary Research Communications*. **e Publication ahead of print, April 2.**

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Reports on the clinico-pathology and mechanisms of trypanosomosis in free-living and captive wild animals showed that clinical disease and outbreaks occur more commonly among captive than free-living wild animals. This is because the free-living wild animals co-exist with the disease until subjected to captivity. In exceptional cases however, drought, starvation and intercurrent diseases often compromised trypanotolerance leading to overt trypanosomosis in free-living wild animals. Meanwhile, in captivity, space restriction, reduced social interactions, change in social herd structure, reduced species-to-species specific behaviours, altered habitat and translocation were the major stressors that precipitated the disease. The cumulative effect of these factors produced severe physiological and somatic stress leading to diminished immune response due to increased blood cortisol output from adrenal cortex. The major symptoms manifested were pyrexia, inappetence,

increased respiration, anaemia, cachexia and death. At necropsy, pulmonary oedema, splenomegaly, hepatomegaly, lymphadenopathy and atrophy of body fats were the gross changes encountered. At the ultra-structural level, the tissues manifested degenerative changes, haemorrhages, necrosis and mononuclear cellular infiltrations. The mechanisms of cellular and tissue injuries were primarily associated with physical and metabolic activities of the organisms. From the foregoing, it is evident that stress is the underlying mechanism that compromises trypanotolerance in wild animals leading to severe clinico-pathological effects.

14848. **Saleh, M. A., Al-Salahy, M. B. & Sanousi, S. A., 2009.** Oxidative stress in blood of camels (*Camelus dromedaries*) naturally infected with *Trypanosoma evansi*. *Veterinary Parasitology*, **162** (3-4): 192-199.

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Oxidative stress is an imbalance between radical-generating and radical-scavenging activity, resulting in oxidation products and tissue damage. The present study aimed to estimate oxidation and antioxidant status in blood of camels naturally infected with *Trypanosoma evansi*. Blood samples from *T. evansi*-infected and healthy (control) female camels were used to determine the free radical nitric oxide (NO) generation in serum, malondialdehyde production in serum (sMDA) and in erythrocytes (eMDA) as a biomarker of lipid peroxidation, blood methaemoglobin formation (MetHb, a biomarker of haemoglobin oxidation), the antioxidants serum ascorbate and albumin levels, erythrocytic glutathione concentration (GSH), superoxide dismutase (SOD) and catalase (CAT) activities. The infected camels were characterized by macrocytic hypochromic anemia. Trypanosomiasis in camels resulted in significant ($p<0.001$) stimulation of serum NO (78.93 percent), eMDA (110.04 percent), sMDA (67.39 percent) and MetHb (1.5-fold) coupled with significant reduction ($p<0.001$) of albumin (27.6 percent), ascorbate (25.38 percent), GSH (43.36 percent), SOD (32.47 percent) and non-significant increase in CAT (7.06 percent, $p=0.322$) compared to control values. In infected camels, a significant positive correlation of NO with eMDA ($r=0.546$, $p=0.009$) and MetHb ($r=0.490$, $p=0.021$) was detected. By contrast, NO was inversely correlated with RBC ($r=-0.546$, $P=0.009$), PCV ($r=-0.427$, $p=0.048$) and Hb ($r=-0.612$, $p=0.002$). On the other hand, eMDA was inversely correlated with RBC ($r=-0.596$, $P=0.003$), PCV ($r=-0.516$, $p=0.014$) and Hb ($r=-0.613$, $p=0.002$). In addition, methaemoglobinemia was negatively correlated with RBC ($r=-0.560$, $p=0.007$), PCV ($r=-0.470$, $p=0.027$) and Hb ($r=-0.585$, $p=0.004$). Our results suggest that chronic *T. evansi* infection in camels is associated with a state of oxidative process.

14849. **Villa, A., Gutierrez, C., Gracia, E., Moreno, B., Chacon, G., Sanz, P. V., Buscher, P. & Touratier, L., 2008.** Presence of *Trypanosoma theileri* in Spanish cattle. *Annals of the New York Academy of Sciences*, **1149**: 352-354.

Exopol Laboratory, Zaragoza, Spain.

Trypanosoma theileri (Laveran, 1902) has been diagnosed in many countries and is commonly considered as a nonpathogenic haemoparasite, although some authors have described clinical signs in cattle infected with *T. theileri*. In April and May, 2005, 12 blood samples were received at the Exopol Diagnostic Laboratory (Zaragoza, Spain) from a

Spanish bull-fighting farm located at Seville province. Clinical exploration of the animals revealed fever, progressive weight loss, anaemia, and frequent recumbent position. Microscopic examination showed *Theileria* spp. in all cases (12), and in four of them, *T. theileri* was also observed. The clinical picture observed in the animals could be compatible with *T. theileri* infection. However, the contribution of *T. theileri* to the clinical signs seen at least in four cases is unknown. Further studies are necessary to determine the pathogenicity of *T. theileri* in the different animal species. To our knowledge, this is the first isolation of *T. theileri* in Spain.

14850. **Watier-Grillot, S., 2008.** Outbreak of animal trypanosomiasis (*T. evansi*) in the Aveyron department of France: risk for implantation of an animal disease with zoonotic potential. *Médecine Tropicale (Mars)*, **68** (5): 468-470.

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No abstract available.

14851. **Welburn, S., Picozzi, K., Coleman, P. G. & Packer, C., 2008.** Patterns in age-seroprevalence consistent with acquired immunity against *Trypanosoma brucei* in Serengeti lions. *PLoS Neglected Tropical Diseases*, **2** (12): e347.

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Trypanosomes cause disease in humans and livestock throughout sub-Saharan Africa. Although various species show evidence of clinical tolerance to trypanosomes, until now there has been no evidence of acquired immunity to natural infections. We discovered a distinct peak and decrease in age prevalence of *T. brucei s.l.* infection in wild African lions that is consistent with being driven by an exposure-dependent increase in cross-immunity following infections with the more genetically diverse species, *T. congolense sensu lato*. The causative agent of human sleeping sickness, *T. brucei rhodesiense*, disappears by 6 years of age apparently in response to cross-immunity from other trypanosomes, including the non-pathogenic subspecies, *T. brucei brucei*. These findings may suggest novel pathways for vaccinations against trypanosomiasis despite the notoriously complex antigenic surface proteins in these parasites.

(c) TRYPANOTOLERANCE

[See also **32**: 14802]

14852. **Berthier, D., Chantal, I., Thevenon, S., Sakande, H., Maillard, J. C., Bengaly, Z., Piquemal, D., Marti, J. & Cuny, G., 2008.** Study of bovine trypanotolerance by whole transcriptome analysis. *Annals of the New York Academy of Sciences*, **1149**: 71-76.

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African trypanosomiasis are parasitic diseases transmitted by tse-tse flies, considered as the main sanitary obstacle to animal production development in sub-Saharan Africa. However, if trypanosomiasis have dramatic consequences on zebu (*Bos indicus*) populations, they have a weaker impact on the western African taurine (*Bos taurus*), which is known to be naturally tolerant to trypanosome infection. Mechanisms governing this trypanotolerant trait are still poorly understood, but today, recent postgenomic biotechnologies, such as the SAGE technique (serial analysis of gene expression) allow us to explore the full transcriptome. Twelve SAGE libraries were constructed from two trypanotolerant animals (N'Dama and Baoulé) and one susceptible species of cattle (the Sudanese zebu) during an experimental *Trypanosoma congolense* infection; 43 458 different tags were obtained at several particular points during the infection (before infection, at the maximum of parasitemia, the maximum of anemia, and at the end of the experiment after value normalization). Bioinformatics analyses highlighted some interesting gene variations with respect to the trypanotolerance status of the animal.

14853. Dayo, G. K., Thevenon, S., Berthier, D., Moazami-Goudarzi, K., Denis, C., Cuny, G., Eggen, A. & Gautier, M., 2009. Detection of selection signatures within candidate regions underlying trypanotolerance in outbred cattle populations. *Molecular Ecology*, **18** (8): 1801-1813.

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Breeding indigenous African taurine cattle tolerant to trypanosomosis is a straightforward approach to control costs generated by this disease. A recent study identified quantitative trait loci (QTL) underlying trypanotolerance traits in experimental crosses between tolerant N'Dama and susceptible Boran zebu cattle. As trypanotolerance is thought to result from local adaptation of indigenous cattle breeds, we propose an alternative and complementary approach to study the genetic architecture of this trait, based on the identification of selection signatures within QTL or candidate genes. A panel of 92 microsatellite markers was genotyped on 509 cattle belonging to four West African trypanotolerant taurine breeds and 10 trypanosusceptible European or African cattle breeds. Some of these markers were located within previously identified QTL regions or candidate genes, while others were chosen in regions assumed to be neutral. A detailed analysis of the genetic structure of these different breeds was carried out to confirm *a priori* grouping of populations based on previous data. Tests based on the comparison of the observed heterozygosities and variances in microsatellite allelic size among trypanotolerant and trypanosusceptible breeds led to the identification of two significantly less variable microsatellite markers. BM4440, one of these two outlier loci, is located within the confidence interval of a previously described QTL underlying a trypanotolerance-related trait. Detection of selection signatures appears to be a straightforward approach for unravelling the molecular determinism of trypanosomosis pathogenesis. We expect that a whole genome approach will help confirm these results and achieve a higher resolving power.

14854. **Geerts, S., Osaer, S., Goossens, B. & Faye, D., 2009.** Trypanotolerance in small ruminants of sub-Saharan Africa. *Trends in Parasitology*, **25** (3): 132-138.

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Although a lot of information is currently available on trypanotolerance in cattle, until recently the trypanotolerant nature of small ruminants was not well known. Trypanotolerance in small ruminants is less pronounced than in cattle and should be considered as resilience rather than resistance. West African Dwarf (WAD) goats seem to be less trypanotolerant than Djallonké sheep. However, recent studies have shown that there is an important introgression of genes of trypanosusceptible breeds into WAD goat populations, which possibly explains the loss of trypanotolerance in these animals. Measures need to be taken to safeguard and upgrade the genetic purity of trypanotolerant goat and sheep breeds in Africa.

14855. **Stein, J., Ayalew, W., Rege, J. E., Mulatu, W., Malmfors, B., Dessie, T. & Philipsson, J., 2009.** Livestock keeper perceptions of four indigenous cattle breeds in tsetse infested areas of Ethiopia. *Tropical Animal Health and Production*. **e Publication ahead of print, February 23.**

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Four cattle breeds indigenous to western and south-western Ethiopia - Abigar, Gurage, Horro and Sheko - were included in a study of the perceptions of smallholder cattle keepers regarding cattle management, production levels and constraints for production. A semi-structured questionnaire was used and 60 cattle keepers from each of the four areas were interviewed. Diseases were reported as the main constraint to cattle production by a majority of livestock keepers in all areas except in the Sheko area, where over-stocking was the main constraint. Among diseases, trypanosomosis was the main livestock disease according to more than half of Gurage, Horro and Sheko keepers, whereas anthrax was most important in the Abigar area. Gurage had highest age at first calving, longest calving interval and also the lowest milk production, whereas Sheko and Abigar had the most favourable characteristics both for milk production (600-700 kg) and fertility (age at first mating 3-3.5 years and above 8 calves/cow). Cattle keepers in the Sheko area reported relatively less problems with cattle diseases compared to the other areas, especially regarding trypanosomosis. Abigar showed a different disease pattern than the other breeds and may also have advantages as regards trypanotolerance.

(d) TREATMENT

14856. **Gutierrez, C., Corbera, J. A., Bayou, K. & van Gool, F., 2008.** Use of cymelarsan in goats chronically infected with *Trypanosoma evansi*. *Annals of the New York Academy of Sciences*, **1149**: 331-333.

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Toxicity and therapeutic trials using Cymelarsan (an arsenical compound) against *Trypanosoma evansi* infection were carried out using chronically infected goats. For the toxicity trial, 40 goats were divided into four groups of 10 animals each; the first three groups received subcutaneous injections of 5, 10, and 15 mg/kg bw of Cymelarsan, respectively, and the last one served as control. No systemic reaction was observed in any goat throughout the experiment. For the therapeutic trial, 15 adult female goats were inoculated intravenously with at least 1×10^5 *T. evansi* isolated in the Canary Islands. Six months after inoculation, the animals were treated with Cymelarsan at single dose of 0.3 mg/kg (5 animals), 0.5 mg/kg (5 animals), and 0.625 mg/kg (5 animals). At 4 and 6 weeks after treatment, two goats belonging to 0.3 mg/kg group showed recurrence of trypanosomes. Parasitaemia, however, was negative in all animals belonging to 0.5 and 0.625 mg/kg groups until the end of the experiment (6 months after treatment). Thus, it can be concluded that Cymelarsan is a safe trypanocidal drug for goats and that the curative dose is 0.5 mg/kg or above.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

[See also **32**: 14820]

14857. **Chiliza, T. E., Van Wyngaardt, W. & Du Plessis, D. H., 2008.** Single-chain antibody fragments from a display library derived from chickens immunized with a mixture of parasite and viral antigens. *Hybridoma*, **27** (6): 413-421.

Immunology Section, ARC-Onderstepoort Veterinary Research Institute, Onderstepoort, Republic of South Africa.

Phage-displayed chicken single-chain antibody fragment libraries can provide useful diagnostic and research reagents. Using avian immunoglobulin genes simplifies the construction of such repertoires since far fewer primer sets are required to access the avian antibody repertoire than is the case with mice or humans. Libraries constructed using mRNA from an immune source are enriched in affinity-matured sequences and consequently need not be as large as "universal" non-immune repertoires to have a reasonable probability of yielding high-affinity binders. Repertoires focused on a number of defined targets can be constructed using lymphocyte mRNA from chickens immunized with a mixture of several

different antigens. This approach was evaluated with the aim of economically and rapidly deriving immunodiagnostic reagents for malaria, trypanosomiasis, and malignant catarrhal fever, all of which are important to health or food security in Africa. Two chickens were each immunized with a mixture comprised of recombinantly expressed histidine-rich protein, the aldolase and the lactate dehydrogenase of *Plasmodium falciparum*, the variant surface glycoprotein of *Trypanosoma* sp., and purified malignant catarrhal fever virus, a herpesvirus that causes an economically important disease of cattle and other ruminants. Immune responses to each of the individual antigens were determined by extracting egg-yolk IgY and testing for antigen-specific antibodies in ELISA. The chicken splenocytes were then recovered, RNA was extracted, and after reverse transcription, the immunoglobulin VH and VL regions were amplified by PCR and joined via a single glycyl residue for surface expression on a collection of filamentous bacteriophages. The resulting display library was then screened by panning to isolate binders. The immunized chickens did not, however, respond equally well to all the different antigens, nor was it possible to derive antibody fragments against all the targets. These limitations notwithstanding, several useful binders with the potential to be used in malaria diagnosis were obtained.

14858. **Thumbi, S. M., McOdimba, F. A., Mosi, R. O. & Jung'a, J. O., 2008.** Comparative evaluation of three PCR base diagnostic assays for the detection of pathogenic trypanosomes in cattle blood. *Parasites and Vectors*, **1** (1): 46.

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Currently, several PCR based diagnostic assays have been developed to improve the detection of pathogenic trypanosomes. These tests include use of species specific primers, single and nested PCRs based on primers amplifying the internal transcribed spacer (ITS) regions of ribosomal DNA. This study compares three PCR based diagnostic assays and assesses the agreement of these three assays by screening 103 cattle blood samples randomly collected from trypanosome endemic areas in western Kenya. The nested ITS based PCR, the single ITS based PCR and the species specific based PCR detected 28.1 percent, 26.2 percent and 10.7 percent of the samples respectively as positive for trypanosome infection. Nested ITS and single ITS PCRs picked 3.8 percent and 1.9 percent as mixed infections respectively. Cohen kappa statistic used to compare agreements beyond chance between the assays showed highest degree of agreement (0.6) between the two ITS based tests, and the lowest (0.2) between the nested PCR test and the species specific PCR. The single ITS and nested ITS based diagnostic assays detected higher numbers of positive cases, and reduced the number of PCR reactions per sample to one and two respectively, compared to the five PCR reactions carried out using the species specific primers. This significantly reduced the labour, time and the cost of carrying out PCR tests, indicating the superiority of the ITS multi-species detection techniques. Reliable epidemiological studies are a prerequisite to designing effective tsetse and trypanosomiasis control programmes. The present study established the suitability of using ITS based PCR assays for large-scale epidemiological studies.

14859. **Tran, T., Claes, F., Verloo, D., De Greve, H. & Buscher, P., 2009.** Towards a new reference test for surra in camels. *Clinical and Vaccine Immunology*. **e Publication ahead of print, April 29.**

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Current serological diagnosis of *Trypanosoma evansi* infection in camels is based on the native variable antigen type RoTat 1.2. The goal of this study is to develop a novel serological diagnostic test based on a non-variable protein and freed from the use of rats or mice for its production. An enzyme-linked immunosorbent assay using recombinant extracellular domain of invariant surface glycoprotein 75 (ELISA/ISG75) was developed and tested on a collection of 184 camel sera. The results were compared to those obtained from three established antibody detection tests based on variable surface glycoprotein RoTat 1.2, *in casu* ELISA/*T. evansi*, card agglutination test for trypanosomiasis (CATT/*T. evansi*) and immune trypanolysis assay (TL). ELISA/ISG75 and ELISA/*T. evansi* showed a sensitivity of 94.6 percent (95 percent confidence interval, CI, 87.8 - 98.2, at 19 percent positivity (PP) cut-off value) and 98.9 percent (95 percent CI 94.1 - 99.8, at 12 PP cut-off value) respectively. ELISA/ISG75 has 100 percent specificity (CI 95.9 - 100), while ELISA/*T. evansi* showed 98.9 percent specificity (CI 95.9 - 100). ELISA/ISG75 demonstrates an almost perfect agreement with TL, CATT/*T. evansi*, and ELISA/*T. evansi*, with Kappa scores of at least 0.94. The ELISA/ISG75 having a comparable performance as the gold standard (TL) and being independent of antigenic variation may become a new reference test for surra in camels. It opens avenues for diagnosis of *T. evansi* infections in other hosts as well as the development of a pan-*Trypanozoon* test for detection of *T. b. brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. evansi* and *T. equiperdum*.

(b) PATHOLOGY AND IMMUNOLOGY

14860. **Boothroyd, C. E., Dreesen, O., Leonova, T., Ly, K. I., Figueiredo, L. M., Cross, G. A. & Papavasiliou, F. N., 2009.** A yeast-endonuclease-generated DNA break induces antigenic switching in *Trypanosoma brucei*. *Nature*, **459**: 278-281.

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Trypanosoma brucei is the causative agent of African sleeping sickness in humans and one of the causes of nagana in cattle. This protozoan parasite evades the host immune

system by antigenic variation, a periodic switching of its variant surface glycoprotein (VSG) coat. VSG switching is spontaneous and occurs at a rate of about 10^{-2} - 10^{-3} per population doubling in recent isolates from nature, but at a markedly reduced rate (10^{-5} - 10^{-6}) in laboratory-adapted strains. VSG switching is thought to occur predominantly through gene conversion, a form of homologous recombination initiated by a DNA lesion that is used by other pathogens (for example, *Candida albicans*, *Borrelia* sp. and *Neisseria gonorrhoeae*) to generate surface protein diversity, and by B lymphocytes of the vertebrate immune system to generate antibody diversity. Very little is known about the molecular mechanism of VSG switching in *T. brucei*. Here we demonstrate that the introduction of a DNA double-stranded break (DSB) adjacent to the approximately 70-base-pair (bp) repeats upstream of the transcribed VSG gene increases switching *in vitro* approximately 250-fold, producing switched clones with a frequency and features similar to those generated early in an infection. We were also able to detect spontaneous DSBs within the 70-bp repeats upstream of the actively transcribed VSG gene, indicating that a DSB is a natural intermediate of VSG gene conversion and that VSG switching is the result of the resolution of this DSB by break-induced replication.

14861. **Chessler, A. D., Ferreira, L. R., Chang, T. H., Fitzgerald, K. A. & Burleigh, B. A., 2008.** A novel IFN regulatory factor 3-dependent pathway activated by trypanosomes triggers IFN-beta in macrophages and fibroblasts. *Journal of Immunology*, **181** (11): 7917-7924.

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Innate immune recognition of intracellular pathogens involves both extracellular and cytosolic surveillance mechanisms. The intracellular protozoan parasite *Trypanosoma cruzi* triggers a robust type 1 IFN response in both immune and non-immune cell types. In this study, we report that signalling through TBK1 and IFN regulatory factor 3 is required for *T. cruzi*-mediated expression of IFN-beta. The TLR adaptors MyD88 and TRIF, as well as TLR4 and TLR3, were found to be dispensable, demonstrating that *T. cruzi* induces IFN-beta expression in a TLR-independent manner. The potential role for cytosolic dsRNA sensing pathways acting through RIG-I and MDA5 was ruled out because *T. cruzi* was shown to trigger robust expression of IFN-beta in macrophages lacking the MAVS/IPS1/VISA/CARDif adaptor protein. The failure of *T. cruzi* to activate HEK293-IFN-beta-luciferase cells, which are highly sensitive to cytosolic triggers of IFN-beta expression including *Listeria*, Sendai virus, and transfected dsRNA and dsDNA, further indicates that the parasite does not engage currently recognized cytosolic surveillance pathways. Together, these findings identify the existence of a novel TLR-independent pathogen-sensing mechanism in immune and nonimmune cells that converges on TBK1 and IFN regulatory factor 3 for activation of IFN-beta gene expression.

14862. **Delgado, M., Anderson, P., Garcia-Salcedo, J. A., Caro, M. & Gonzalez-Rey, E., 2009.** Neuropeptides kill African trypanosomes by targeting intracellular compartments and inducing autophagic-like cell death. *Cell Death and Differentiation*, **16** (3): 406-416.

Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada, Spain.

Trypanosoma brucei is the causative agent of African sleeping sickness. Available treatments are ineffective, toxic and susceptible to resistance by the parasite. Here we show that various endogenous neuropeptides act as potent antitrypanosome agents. Neuropeptides exerted their trypanolytic activity through an unusual mechanism that involves peptide uptake by the parasite, disruption of lysosome integrity and cytosolic accumulation of glycolytic enzymes. This promotes an energetic metabolism failure that initiates an autophagic-like cell death. Neuropeptide-based treatment improved clinical signs in a chronic model of trypanosomiasis by reducing the parasite burden in various target organs. Of physiological importance is the fact that hosts respond to trypanosome infection producing neuropeptides as part of their natural innate defence. From a therapeutic point of view, targeting of intracellular compartments by neuropeptides provides a new promising strategy for the treatment of trypanosomiasis.

14863. **Fatih, M. Y., Adamu, S., Umar, I. A., Ibrahim, N. D., Eduvie, L. O. & Esievo, K. A., 2008.** Studies on effects of lactose on experimental *Trypanosoma vivax* infection in Zebu cattle. 2. Packed cell volume. *Onderstepoort Journal of Veterinary Research*, **75** (3): 181-187.

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The ability of intravenously administered lactose in normal saline to prevent a decline in packed cell volume (PCV) during experimental trypanosomosis was studied in Zebu cattle. During the lactose infusion period, the PCV was stable up to day 5 post-infection (p.i.) in a lactose-infused group, compared to that in an uninfused group in which the PCV dropped significantly ($p < 0.05$) as shown by the values of cumulative percentage change. Furthermore the mean rate of change in PCV was significantly ($p < 0.05$) higher in the uninfused group relative to the lactose-infused group during the same period. While the PCV fell markedly in the lactose-infused group a day after lactose infusion was stopped (day 13 p.i.), subsequent PCV values were significantly ($p < 0.05$) higher compared to those in the uninfused group, up to the end of experiment on day 17 p.i. However the mean rates of change in PCV did not vary significantly ($p > 0.05$) between the groups during the period in which lactose infusion was stopped. The mean levels of parasitaemic waves and parasitaemia were higher, more prolonged and more frequent in the lactose-infused group. It was inferred that the lactose was able to prevent an early onset of anaemia in the *Trypanosoma vivax*-infected Zebu cattle.

14864. **Grebaut, P., Chuchana, P., Brizard, J. P., Demettre, E., Seveno, M., Bossard, G., Jouin, P., Vincendeau, P., Bengaly, Z., Boulange, A., Cuny, G. & Holzmüller, P., 2009.** Identification of total and differentially expressed excreted-secreted proteins from *Trypanosoma congolense* strains exhibiting different virulence and pathogenicity. *International Journal of Parasitology*. **e Publication ahead of print March 13.**

CIRAD UMR 17 Trypanosomes [UMR 177 IRD-CIRAD "Interactions Hôtes-Vecteurs-Parasites dans les Trypanosomoses"], TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.

Animal trypanosomosis is a major constraint to livestock productivity in the tropics and has a significant impact on the life of millions of people globally (mainly in Africa, South America and south-east Asia). In Africa, the disease in livestock is caused mainly by *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma evansi* and *Trypanosoma brucei brucei*. The extracellular position of trypanosomes in the bloodstream of their host requires consideration of both the parasite and its naturally excreted-secreted factors (secretome) in the course of pathophysiological processes. We therefore developed and standardised a method to produce purified proteomes and secretomes of African trypanosomes. In this study, two strains of *T. congolense* exhibiting opposite properties of both virulence and pathogenicity were further investigated through their secretome expression and its involvement in host-parasite interactions. We used a combined proteomic approach (one-dimensional SDS-PAGE and two-dimensional differential in-gel electrophoresis coupled to mass spectrometry) to characterise the whole and differentially expressed protein contents of secretomes. The molecular identification of differentially expressed trypanosome molecules and their correlation with either the virulence process or pathogenicity are discussed with regard to their potential as new diagnostic or therapeutic tools against animal trypanosomosis.

14865. **Guilliams, M., Movahedi, K., Bosschaerts, T., VandenDriessche, T., Chuah, M. K., Herin, M., Acosta-Sanchez, A., Ma, L., Moser, M., Van Ginderachter, J. A., Brys, L., De Baetselier, P. & Beschin, A., 2009.** IL-10 dampens TNF/inducible nitric oxide synthase-producing dendritic cell-mediated pathogenicity during parasitic infection. *Journal of Immunology*, **182** (2): 1107-1118.

Department of Molecular and Cellular Interactions, VIB, Brussels, Belgium.

Antiparasite responses are associated with the recruitment of monocytes that differentiate to macrophages and dendritic cells at the site of infection. Although classically activated monocytic cells are assumed to be the major source of TNF and NO during *Trypanosoma brucei brucei* infection, their cellular origin remains unclear. In this study, we show that bone marrow-derived monocytes accumulate and differentiate to TNF/inducible NO synthase-producing dendritic cells (TIP-DCs) in the spleen, liver, and lymph nodes of *T. brucei brucei*-infected mice. Although TIP-DCs have been shown to play a beneficial role in the elimination of several intracellular pathogens, we report that TIP-DCs, as a major source of TNF and NO in inflamed organs, could contribute actively to tissue damage during the chronic stage of *T. brucei brucei* infection. In addition, the absence of IL-10 leads to enhanced differentiation of monocytes to TIP-DCs, resulting in exacerbated pathogenicity and early death of the host. Finally, we demonstrate that sustained production of IL-10 following IL-10 gene delivery treatment with an adeno-associated viral vector to chronically infected mice limits the differentiation of monocytes to TIP-DCs and protects the host from tissue damage.

14866. **Kibugu, J. K., Ngeranwa, J. J., Makumi, J. N., Gathumbi, J. K., Kagira, J. M., Mwangi, J. N., Muchiri, M. W. & Mdachi, R. E., 2009.** Aggravation of pathogenesis mediated by ochratoxin A in mice infected with *Trypanosoma brucei rhodesiense*. *Parasitology*, **136** (3): 273-281.

Kenya Agricultural Research Institute, Trypanosomiasis Research Centre, P. O. Box 362, Kikuyu, Kenya. [jkkibugu@yahoo.com].

Mice fed 1.5 mg ochratoxin A (OTA) per kg body weight and infected with *Trypanosoma brucei rhodesiense* were compared with trypanosome-infected placebo-fed and uninfected OTA-fed controls. Uninfected OTA-fed mice showed fever, lethargy, facial and eyelid oedemas, mild hepatitis and nephritis, and high survival. Infected placebo-fed controls had mean pre-patent period (PPP) of 3.26 days, lethargy, dyspnoea, fever, facial and scrotal oedema, survival of 33-65 days, reduced red cell counts (RCC: $10.96-6.87 \times 10^6$ cells/ μ L of blood), packed cell volume (PCV: 43.19-26.36 percent), haemoglobin levels (Hb: 13.37-7.92 g/dL) and mean corpuscular volume (MCV) of 37.96-41.31 fL, hepatosplenomegaly, generalized oedemas, heart congestion, hepatitis and nephritis. Compared to infected placebo-fed controls, infected OTA-fed mice had significantly ($p < 0.05$) shorter mean PPP (2.58 days), reduced survival (6-47 days), more pronounced fever and dyspnoea. The latter had significantly ($p < 0.05$) reduced RCC ($10.74-4.56 \times 10^6$ cells/ μ L of blood), PCV (43.90-20.78 percent), Hb (13.06-5.74 g/dL), increased MCV (39.10-43.97 fL), severe generalized oedemas, haemorrhages, congestion, hepatic haemosiderosis, hepatitis, nephritis, endocarditis, pericarditis and exclusively, splenic macrophage and giant cell hyperplasia, expanded red pulp and splenic erythrophagocytosis. It was concluded that OTA aggravated the pathogenesis of *T. b. rhodesiense* infection in mice, and should therefore be taken into consideration during trypanosomiasis control programmes.

14867. **Margolles-Clark, E., Jacques-Silva, M. C., Ganesan, L., Umland, O., Kenyon, N. S., Ricordi, C., Berggren, P. O. & Buchwald, P., 2009.** Suramin inhibits the CD40-CD154 co-stimulatory interaction: a possible mechanism for immunosuppressive effects. *Biochemical Pharmacology*, **77** (7): 1236-1245.

Diabetes Research Institute, Miller School of Medicine, University of Miami, FL, USA.

Suramin is a symmetric polysulphonated naphthylamine-benzamide urea derivative approved for the treatment of trypanosomiasis and onchocerciasis and a known P2 (ATP/UTP purine receptor) antagonist. Here, we report its ability to inhibit the important CD40-CD154 co-stimulatory interaction required for T cell activation and the development of an effective immune response. *In vitro*, it inhibited the binding of both human and murine CD154 (CD40L) to their receptor (CD40) even in the presence of protein-containing media and prevented the CD154-induced proliferation of human B cells as well as the corresponding increase in surface expression of CD86, CD80, CD40, and MHC class II in a concentration-dependent manner. Furthermore, in isolated human islets, it also decreased the CD154-induced release of inflammatory cytokines such as IFN- γ , interleukin-6 (IL-6), and IL-8. Suramin was selected for investigation because it has been reported to be an inhibitor of the interaction of TNF- α with its receptor and CD154 is a member of the TNF family. However, it turned out to be a considerably, about 30-fold, more effective inhibitor of the CD40-CD154

protein-protein interaction than of the corresponding TNF interaction. Its median inhibitory concentration (IC₅₀ 50 mM) is somewhat higher than for the P2-receptor, but well within the range of its therapeutic concentration levels. Suramin shows considerable polypharmacology, but its interference with the positive co-stimulatory interaction might provide a possible, not yet identified mechanism for its ability to suppress T cell activity and induce immunosuppression, which might also have limited its clinical usefulness in the treatment of AIDS and cancer.

14868. **Namangala, B., De Baetselier, P. & Beschin, A., 2009.** Both type-I and type-II responses contribute to murine trypanotolerance. *Journal of Veterinary Medical Science*, **71** (3): 313-318.

The University of Zambia, Faculty of Veterinary Medicine, Department of Paraclinical Studies, Lusaka, Zambia. [boniface_1020@yahoo.com].

The host immune system has been documented to influence the course and outcome of infection with the phospholipase-C-deficient (PLC(-/-)) *Trypanosoma brucei brucei*. We addressed the resistance mechanisms during trypanosomosis by comparing the immune response to variant-specific surface glycoprotein (VSG) in relatively susceptible C3H mice and trypanotolerant (C57BL/6 x BALB/c)-F1 (B6B-F1) mice infected with PLC(-/-) parasites. During the early stage of infection, lymphoid cells from both PLC(-/-)-susceptible C3H and -tolerant B6B-F1 mice mainly secreted VSG-specific IFN-gamma. Although C3H mice remained locked in a type-I cytokine environment (IFN-gamma, TNF-alpha) during late stage of infection, B6B-F1 mice switched to production of type-II cytokines (IL-4, IL-10) from late stage of infection onwards. It seems that VSG-specific cytokine responses associated with resistance to murine African trypanosomosis are infection-stage dependent, with type-I cytokine responses being critical during the early stage of infection while type-II cytokine responses appear to be more important during the late and chronic phases of the disease. Because of the striking similarities in the course of the PLC(-/-)-infection in B6B-F1 mice with that of the trypanotolerant N'Dama cattle naturally-infected with *T. congolense*, the PLC(-/-)-infected B6B-F1 mice represents a suitable model to study the course of infection and immune responses during bovine trypanosomosis.

14869. **Ngure, R. M., Eckersall, P. D., Mungatana, N. K., Mburu, J. N., Jennings, F. W., Burke, J. & Murray, M., 2009.** Lipopolysaccharide binding protein in the acute phase response of experimental murine *Trypanosoma brucei brucei* infection. *Research in Veterinary Science*, **86** (3): 394-398.

Department of Veterinary Medicine, University of Glasgow, Bearsden Road, Bearsden, Glasgow G61 1QH, UK; Department of Biochemistry and Molecular Biology, Egerton University, P.O. Box 536, Egerton, Kenya.

Cellular responses to lipopolysaccharide (LPS) are enhanced by LPS-binding protein (LBP). The present study investigated the acute phase response of LBP during *Trypanosoma brucei brucei* infection in mice. Mean plasma concentrations of LBP increased two-fold by the seventh day following infection, but decreased to intermediate levels by the 14th day. There were no significant differences in LBP concentrations of infected/antibiotic-treated and infected/untreated mice. At 35 days post-infection, the infected mice were treated with the

anti-trypanosomal diminazine aceturate (Berenil). LBP levels of the mice then decreased to pre-infection levels within one-week. This demonstrated that LBP is an acute phase protein during murine trypanosomosis. Furthermore, opportunistic secondary bacterial infection during trypanosomosis did not seem to play an important role in the changes in plasma LBP levels. We speculate that the marked concomitant increases in plasma LBP and endotoxin-like activity following murine trypanosome infection might play an important role in the pathogenesis of trypanosomosis.

14870. **Oliveira, M. P., Cortez, M., Maeda, F. Y., Fernandes, M. C., Haapalainen, E. F., Yoshida, N. & Mortara, R. A., 2009.** Unique behaviour of *Trypanosoma dionisii* interacting with mammalian cells: invasion, intracellular growth, and nuclear localization. *Acta Tropica*, **110** (1): 65-74.

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The phylogenetic proximity between *Trypanosoma cruzi* and *Trypanosoma (Schizotrypanum) dionisii* suggests that these parasites might explore similar strategies to complete their life cycles. *T. cruzi* is the aetiological agent of the life-threatening Chagas disease, whereas *T. dionisii* is a bat trypanosome and probably not capable of infecting humans. Here we sought to compare mammalian cell invasion and intracellular traffic of both trypanosomes and determine the differences and similarities in this process. The results presented demonstrate that *T. dionisii* is highly infective *in vitro*, particularly when the infection process occurs without serum and that the invasion is similarly affected by agents known to interfere with *T. cruzi* invasion process. Our results indicate that the formation of lysosomal-enriched compartments is part of a cell-invasion mechanism retained by related trypanosomatids, and that residence and further escape from a lysosomal compartment may be a common requisite for successful infection. During intracellular growth, parasites share a few epitopes with *T. cruzi* amastigotes and trypomastigotes. Unexpectedly, in heavily infected cells, amastigotes and trypomastigotes were found inside the host cell nucleus. These findings suggest that *T. dionisii*, although sharing some features in host cell invasion with *T. cruzi*, has unique behaviours that deserve to be further explored.

14871. **Rodgers, J., Stone, T. W., Barrett, M. P., Bradley, B. & Kennedy, P. G., 2009.** Kynurenine pathway inhibition reduces central nervous system inflammation in a model of human African trypanosomiasis. *Brain*. **Advance Access published on line March 31.**

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Human African trypanosomiasis, or sleeping sickness, is caused by the protozoan parasites *Trypanosoma brucei rhodesiense* or *Trypanosoma brucei gambiense*, and is a major cause of systemic and neurological disability throughout sub-Saharan Africa. Following early-stage disease, the trypanosomes cross the blood-brain barrier to invade the central nervous system leading to the encephalitic, or late stage, infection. Treatment of human African trypanosomiasis currently relies on a limited number of highly toxic drugs, but untreated, is invariably fatal. Melarsoprol, a trivalent arsenical, is the only drug that can be

used to cure both forms of the infection once the central nervous system has become involved, but unfortunately, this drug induces an extremely severe post-treatment reactive encephalopathy (PTRE) in up to 10 percent of treated patients, half of whom die from this complication. Since it is unlikely that any new and less toxic drug will be developed for treatment of human African trypanosomiasis in the near future, increasing attention is now being focussed on the potential use of existing compounds, either alone or in combination chemotherapy, for improved efficacy and safety. The kynurenine pathway is the major pathway in the metabolism of tryptophan. A number of the catabolites produced along this pathway show neurotoxic or neuroprotective activities, and their role in the generation of central nervous system inflammation is well documented. In the current study, Ro-61-8048, a high affinity kynurenine-3-monooxygenase inhibitor, was used to determine the effect of manipulating the kynurenine pathway in a highly reproducible mouse model of human African trypanosomiasis. It was found that Ro-61-8048 treatment had no significant effect ($P = 0.4445$) on the severity of the neuroinflammatory pathology in mice during the early central nervous system stage of the disease when only a low level of inflammation was present. However, a significant ($p = 0.0284$) reduction in the severity of the neuroinflammatory response was detected when the inhibitor was administered in animals exhibiting the more severe, late central nervous system stage, of the infection. *In vitro* assays showed that Ro-61-8048 had no direct effect on trypanosome proliferation suggesting that the anti-inflammatory action is due to a direct effect of the inhibitor on the host cells and not a secondary response to parasite destruction. These findings demonstrate that kynurenine pathway catabolites are involved in the generation of the more severe inflammatory reaction associated with the late central nervous system stages of the disease and suggest that Ro-61-8048 or a similar drug may prove to be beneficial in preventing or ameliorating the PTRE when administered as an adjunct to conventional trypanocidal chemotherapy.

14872. **Samanovic, M., Molina-Portela, M. P., Chessler, A. D., Burleigh, B. A. & Raper, J., 2009.** Trypanosome lytic factor, an antimicrobial high-density lipoprotein, ameliorates *Leishmania* infection. *PLoS Pathogens*, **5** (1): e1000276.

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Innate immunity is the first line of defence against invading microorganisms. Trypanosome lytic factor (TLF) is a minor sub-fraction of human high-density lipoprotein that provides innate immunity by completely protecting humans from infection by most species of African trypanosomes, which belong to the Kinetoplastida order. Herein, we demonstrate the broader protective effects of human TLF, which inhibits intracellular infection by *Leishmania*, a kinetoplastid that replicates in phagolysosomes of macrophages. We show that TLF accumulates within the parasitophorous vacuole of macrophages *in vitro* and reduces the number of *Leishmania* metacyclic promastigotes, but not amastigotes. We do not detect any activation of the macrophages by TLF in the presence or absence of *Leishmania*, and therefore propose that TLF directly damages the parasite in the acidic parasitophorous vacuole. To investigate the physiological relevance of this observation, we have reconstituted lytic activity *in vivo* by generating mice that express the two main protein components of TLFs: human apolipoprotein L-I and haptoglobin-related protein. Both proteins are expressed in mice at levels equivalent to those found in humans and circulate within high-density lipoproteins. We find that TLF mice can ameliorate an infection with

Leishmania by significantly reducing the pathogen burden. In contrast, TLF mice were not protected against infection by the kinetoplastid *Trypanosoma cruzi*, which infects many cell types and transiently passes through a phagolysosome. We conclude that TLF not only determines species specificity for African trypanosomes, but can also ameliorate an infection with *Leishmania*, while having no effect on *T. cruzi*. We propose that TLFs are a component of the innate immune system that can limit infections by their ability to selectively damage pathogens in phagolysosomes within the reticuloendothelial system.

(c) CHEMOTHERAPEUTICS

14873. **Alloatti, A., Testero, S. A. & Uttaro, A. D., 2009.** Chemical evaluation of fatty acid desaturases as drug targets in *Trypanosoma cruzi*. *International Journal of Parasitology*. **In press, corrected proof.**

Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET, Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmaceuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Santa Fe, Argentina.

Four positional isomers of thiastearate (TS) and isoxyl (thiocarlide) were assayed as fatty acid desaturase inhibitors in *Trypanosoma cruzi* epimastigotes. 9-TS did not exert a significant effect on growth of *T. cruzi*, nor on the fatty acid profile of the parasite cells. One hundred μM of 10-TS totally inhibited growth, with an effective concentration for 50 percent growth inhibition (EC(50)) of $3.0 \pm 0.2 \mu\text{M}$. Growth inhibition was reverted by supplementing the culture media with oleate. The fatty acid profile of treated cells revealed that conversion of stearate to oleate and palmitate to palmitoleate were drastically reduced and, as a consequence, the total level of unsaturated fatty acids decreased from 60 percent to 32 percent. Isoxyl, a known inhibitor of stearyl-CoA Delta9 desaturase in mycobacteria, had similar effects on *T. cruzi* growth (EC(50) $2.0 \pm 0.3 \mu\text{M}$) and fatty acid content, indicating that Delta9 desaturase was the target of both drugs. 12- and 13-TS were inhibitors of growth with EC(50) values of 50 ± 2 and $10 \pm 3 \mu\text{M}$, respectively, but oleate or linoleate were unable to revert the effect. Both drugs increased the percentage of oleate and palmitate in the cell membrane and drastically reduced the content of linoleate from 38 percent to 16 percent and 12 percent, respectively, which is in agreement with a specific inhibition of oleate Delta12 desaturase. The absence of corresponding enzyme activity in mammalian cells and the significant structural differences between trypanosome and mammalian Delta9 desaturases, together with our results, highlight these enzymes as promising targets for selective chemotherapeutic intervention.

14874. **Bakunova, S. M., Bakunov, S. A., Patrick, D. A., Kumar, E. V., Ohemeng, K. A., Bridges, A. S., Wenzler, T., Barszcz, T., Jones, S. K., Werbovetz, K. A., Brun, R. & Tidwell, R. R., 2009.** Structure-activity study of pentamidine analogues as antiprotozoal agents. *Journal of Medicinal Chemistry*, **52** (7): 2016-2035.

Department of Pathology and Laboratory Medicine, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina 27599, USA.

Diamidine 1 (pentamidine) and 65 analogues (2-66) have been tested for *in vitro* antiprotozoal activities against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani*, and for cytotoxicity against mammalian cells. Dications 32, 64, and 66 exhibited antitrypanosomal potencies equal or greater than melarsoprol (IC(50) = 4 nM). Nine congeners (2-4, 12, 27, 30, and 64-66) were more active against *P. falciparum* than artemisinin (IC(50) = 6 nM). Eight compounds (12, 32, 33, 44, 59, 62, 64, and 66) exhibited equal or better antileishmanial activities than 1 (IC(50) = 1.8 μ M). Several congeners were more active than 1 *in vivo*, curing at least 2/4 infected animals in the acute mouse model of trypanosomiasis. The diimidazoline 66 was the most promising compound in the series, showing excellent *in vitro* activities and high selectivities against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* combined with high antitrypanosomal efficacy *in vivo*.

14875. **Baliani, A., Peal, V., Gros, L., Brun, R., Kaiser, M., Barrett, M. P. & Gilbert, I. H., 2009.** Novel functionalized melamine-based nitroheterocycles: synthesis and activity against trypanosomatid parasites. *Organic and Biomolecular Chemistry*, **7** (6): 1154-1166.

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Human African trypanosomiasis (HAT), caused by the protozoan parasite *Trypanosoma brucei* sp., is a major health problem in sub-Saharan Africa. New drugs are urgently required for the disease. Selective uptake of toxic compounds into trypanosomes has been achieved by exploiting plasma membrane transporters. For example, the P2 aminopurine transporter, along with other transporters, selectively concentrates melamine and benzamidine moieties into trypanosomes. We have previously reported the use of the melamine motif to selectively target nitrofurantoin to the trypanosome. In this paper we report the further investigation of the structure activity relationships and the effect of the introduction of different functionalized substituents onto the melamine unit. Most of the compounds tested *in vitro* for their trypanocidal activity showed activities in the sub μ M range against *T. b. rhodesiense*.

14876. **Barker, R. H., Jr., Liu, H., Hirth, B., Celatka, C. A., Fitzpatrick, R., Xiang, Y., Willert, E. K., Phillips, M. A., Kaiser, M., Bacchi, C. J., Rodriguez, A., Yarlett, N., Klinger, J. D. & Sybertz, E., 2009.** Novel S-adenosylmethionine decarboxylase inhibitors for the treatment of human African trypanosomiasis. *Antimicrobial Agents and Chemotherapy*, **53** (5): 2052-2058.

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Trypanosomiasis remains a significant disease across the sub-Saharan African continent, with 50 000 to 70 000 individuals infected. The utility of current therapies is limited by issues of toxicity and the need to administer compounds intravenously. We have begun a program to pursue lead optimization around MDL 73811, an irreversible inhibitor of

S-adenosylmethionine decarboxylase (AdoMetDC). This compound is potent but in previous studies cleared rapidly from the blood of rats. One of the analogues synthesized (Genz-644131) was shown to be highly active against *Trypanosoma brucei rhodesiense* *in vitro* (50 percent inhibitory concentration, 400 pg/ml). Enzyme kinetic studies showed Genz-644131 to be approximately fivefold more potent than MDL 73811 against the *T. brucei brucei* AdoMetDC-prozyme complex. This compound was stable *in vitro* in rat and human liver microsomal and hepatocyte assays, was stable in rat whole-blood assays, did not significantly inhibit human cytochrome P450 enzymes, had no measurable efflux in CaCo-2 cells, and was only 41 percent bound by serum proteins. Pharmacokinetic studies of mice following intraperitoneal dosing showed that the half-life of Genz-644131 was threefold greater than that of MDL 73811 (7.4 h versus 2.5 h). Furthermore, brain penetration of Genz-644131 was 4.3-fold higher than that of MDL 73811. Finally, *in vivo* efficacy studies of *T. b. brucei* strain STIB 795-infected mice showed that Genz-644131 significantly extended survival (from 6.75 days for controls to >30 days for treated animals) and cured animals infected with *T. b. brucei* strain LAB 110 EATRO. Taken together, the data strengthen validation of AdoMetDC as an important parasite target, and these studies have shown that analogues of MDL 73811 can be synthesized with improved potency and brain penetration.

14877. **Benitez, J., Guggeri, L., Tomaz, I., Arrambide, G., Navarro, M., Pessoa, J. C., Garat, B. & Gambino, D., 2009.** Design of vanadium mixed-ligand complexes as potential antiprotozoa agents. *Journal of Inorganic Biochemistry*, **103** (4): 609-616.

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In the search for new therapeutic tools against Chagas' disease (American Trypanosomiasis) four novel mixed-ligand vanadyl complexes, [V(IV)O(L(2)-2H)(L(1))], including a bidentate polypyridyl DNA intercalator (L(1)) and a tridentate salicylaldehyde semicarbazone derivative (L(2)) as ligands were synthesized, characterized by a combination of techniques, and *in vitro* evaluated. EPR suggest a distorted octahedral geometry with the tridentate semicarbazone occupying three equatorial positions and the polypyridyl ligand coordinated in an equatorial/axial mode. Both complexes including dipyrro[3,2-a: 2',3'-c]phenazine (dppz) as polypyridyl coligand showed IC(50) values in the μM range against Dm28c strain (epimastigotes) of *Trypanosoma cruzi*, causative agent of the disease, being as active as the anti-trypanosomal reference drug Nifurtimox. To get an insight into the trypanocidal mechanism of action of these compounds, DNA was evaluated as a potential parasite target and EPR, and (51)V NMR experiments were also carried out upon aging aerated solutions of the complexes. Data obtained by electrophoretic analysis suggest that the mechanism of action of these complexes could include DNA interactions.

14878. **Cameron, S., Martini, V. P., Iulek, J. & Hunter, W. N., 2009.** *Geobacillus stearothermophilus* 6-phosphogluconate dehydrogenase complexed with 6-phosphogluconate. *Acta Crystallographia Section F Structural Biology and Crystalization Communications*, **65** (Pt 5): 450-454.

University of Dundee, Scotland, UK.

Two crystal structures of recombinant *Geobacillus stearothermophilus* 6-phosphogluconate dehydrogenase (Gs6PDH) in complex with the substrate 6-phosphogluconate have been determined at medium resolution. Gs6PDH shares significant sequence identity and structural similarity with the enzymes from *Lactococcus lactis*, sheep liver and the protozoan parasite *Trypanosoma brucei*, for which a range of structures have previously been reported. Comparisons indicate that amino-acid sequence conservation is more pronounced in the two domains that contribute to the architecture of the active site, namely the N-terminal and C-terminal domains, compared with the central domain, which is primarily involved in the subunit-subunit associations required to form a stable dimer. The active-site residues are highly conserved, as are the interactions with the 6-phosphogluconate. There is interest in 6PDH as a potential drug target for the protozoan parasite *T. brucei*, the pathogen responsible for African sleeping sickness. The recombinant *T. brucei* enzyme has proven to be recalcitrant to enzyme-ligand studies and a surrogate protein might offer new opportunities to investigate and characterize 6PDH inhibitors. The high degree of structural similarity, efficient level of expression and straightforward crystallization conditions mean that Gs6PDH may prove to be an appropriate model system for structure-based inhibitor design targeting the enzyme from *Trypanosoma* species.

14879. **Chauhan, S. C., Padmanabhan, P. K. & Madhubala, R., 2008.** Glyoxalase pathway of trypanosomatid parasites: a promising chemotherapeutic target. *Current Drug Targets*, **9** (11): 957-965.

School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India.

Trypanosomatids are pathogenic protozoa of the order Kinetoplastida. A unique feature of these parasitic protozoa is the presence of a unique dithiol trypanothione (N(1), N(8) -bis(glutathionyl)spermidine) and the flavoenzyme trypanothione reductase. This is in contrast to human and other eukaryotes, which contain ubiquitous glutathione/glutathione reductase system. An important function of thiols is to protect cells from toxic metabolic by-products such as methylglyoxal, a reactive 2-oxoaldehyde. Methylglyoxal is a mutagenic and a cytotoxic compound. The glyoxalase system is involved in the detoxification of methylglyoxal. The exceptionality of the glyoxalase enzyme in the parasitic protozoa is the dependence on the dithiol -trypanothione for detoxifying the toxic methylglyoxal. The detoxification process by the glyoxalase enzyme in eukaryotes and most other organisms is dependent on the tripeptide glutathione. The glyoxalase enzyme of trypanosomatids are also exceptional in a way that they use the divalent cation nickel as a cofactor like the glyoxalase enzyme of *E. coli*, whereas in eukaryotes the cofactor is zinc. This reflects that both the substrate as well as the cofactor of the kinetoplastids glyoxalase enzyme are distinct from that of the glyoxalase enzyme of eukaryotes. These differences reveal that the active site of the glyoxalase enzyme of the parasite and its mammalian counterpart are significantly different thereby proposing that the glyoxalase enzyme of the protozoan parasite can be a potential chemotherapeutic target.

14880. **Chollet, C., Baliani, A., Wong, P. E., Barrett, M. P. & Gilbert, I. H., 2009.** Targeted delivery of compounds to *Trypanosoma brucei* using the melamine motif. *Bioorganic and Medicinal Chemistry*, **17** (6): 2512-2523.

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There is an urgent need for the development of new drugs for the treatment of human African trypanosomiasis. The causative organism, *Trypanosoma brucei*, has been shown to have some unusual plasma membrane transporters, in particular the P2 aminopurine transporter and related permeases, which have been used for the selective targeting of trypanocidal compounds to the organism. In this paper, we report the addition of melamine-based P2-targeting motifs to three different classes of compound in order to try and improve activity through increased selective uptake. The classes reported here are fluoroquinolones, difluoromethylornithine and artesunate derivatives.

14881. **Field, M. C., 2009.** Drug screening by crossing membranes: a novel approach to identification of trypanocides. *Biochemical Journal*, **419** (2): e1-3.

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Trypanosomes are a group of protozoan parasites that inflict huge health and economic burdens across the globe. The African trypanosome, *Trypanosoma brucei*, the causative agent of sleeping sickness, has a highly sophisticated mechanism of antigenic variation that facilitates chronic survival in the mammalian host, and also all but eliminates any realistic hope for vaccination-based control. However, trypanosomes are also highly divergent organisms, with many biochemical processes setting them apart from their hosts, and there remains great optimism that these features may be exploited for development of new drugs. Unfortunately, the compounds that are in use at present are decades old and resistance has emerged. The article in this issue of the *Biochemical Journal* by Patham *et al.*, a joint team from the universities of Pittsburgh and Georgia, represents one approach to exploiting this divergence. The authors of the study have exploited novel aspects of the biochemistry within the system for translocation of nascent polypeptides across the endoplasmic reticulum membrane to identify three compounds that are able to inhibit the process. They then demonstrate that these same compounds are both trypanocidal, but well tolerated by human tissue culture cells. These observations may present interesting new leads in the fight against trypanosomiasis, and potentially identify a new target that can be explored for therapeutic potential.

14882. **Haines, L. R., Thomas, J. M., Jackson, A. M., Eyford, B. A., Razavi, M., Watson, C. N., Gowen, B., Hancock, R. E. & Pearson, T. W., 2009.** Killing of trypanosomatid parasites by a modified bovine host defence peptide, BMAP-18. *PLoS Neglected Tropical Diseases*, **3** (2): e373.

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Tropical diseases caused by parasites continue to cause socioeconomic devastation that reverberates worldwide. There is a growing need for new control measures for many of these diseases due to increasing drug resistance exhibited by the parasites and problems with drug toxicity. One new approach is to apply host defence peptides (HDP; formerly called antimicrobial peptides) to disease control, either to treat infected hosts, or to prevent disease transmission by interfering with parasites in their insect vectors. A potent anti-parasite effector is bovine myeloid antimicrobial peptide-27 (BMAP-27), a member of the cathelicidin family. Although BMAP-27 is a potent inhibitor of microbial growth, at higher concentrations it also exhibits cytotoxicity to mammalian cells. We tested the anti-parasite activity of BMAP-18, a truncated peptide that lacks the hydrophobic C-terminal sequence of the BMAP-27 parent molecule, an alteration that confers reduced toxicity to mammalian cells. BMAP-18 showed strong growth inhibitory activity against several species and life cycle stages of African trypanosomes, fish trypanosomes and *Leishmania* parasites *in vitro*. When compared to native BMAP-27, the truncated BMAP-18 peptide showed reduced cytotoxicity on a wide variety of mammalian and insect cells and on *Sodalis glossinidius*, a bacterial symbiont of the tsetse vector. The fluorescent stain rhodamine 123 was used in immunofluorescence microscopy and flow cytometry experiments to show that BMAP-18 at low concentrations rapidly disrupted mitochondrial potential without obvious alteration of parasite plasma membranes, thus inducing death by apoptosis. Scanning electron microscopy revealed that higher concentrations of BMAP-18 induced membrane lesions in the parasites as early as 15 min. after exposure, thus killing them by necrosis. In addition to direct killing of parasites, BMAP-18 was shown to inhibit LPS-induced secretion of tumour necrosis factor alpha (TNF-alpha), a cytokine that is associated with inflammation and cachexia (wasting) in sleeping sickness patients. As a prelude to *in vivo* applications, high affinity antibodies to BMAP-18 were produced in rabbits and used in immuno-mass spectrometry assays to detect the intact peptide in human blood and plasma. In conclusion, BMAP-18, a truncated form of the potent antimicrobial BMAP-27, showed low toxicity to mammalian cells, insect cells and the tsetse bacterial symbiont *Sodalis glossinidius* while retaining an ability to kill a variety of species and life cycle stages of pathogenic kinetoplastid parasites *in vitro*. BMAP-18 also inhibited secretion of TNF-alpha, an inflammatory cytokine that plays a role in the cachexia associated with African sleeping sickness. These findings support the idea that BMAP-18 should be explored as a candidate for therapy of economically important trypanosome-infected hosts, such as cattle, fish and humans, and for paratransgenic expression in *Sodalis glossinidius*, a bacterial symbiont in the tsetse vector, as a strategy for interference with trypanosome transmission.

14883. Ishiyama, A., Otoguro, K., Namatame, M., Nishihara, A., Furusawa, T., Masuma, R., Shiomi, K., Takahashi, Y., Ichimura, M., Yamada, H. & Omura, S., 2008. *In vitro* and *in vivo* antitrypanosomal activity of two microbial metabolites, KS-505a and alazopeptin. *Journal of Antibiotics (Tokyo)*, **61** (10): 627-632.

Research Center for Tropical Diseases, Center for Basic Research, Kitasato University, Tokyo, Japan.

Our on-going screening programme to discover new antitrypanosomal antibiotics has been evaluating compounds isolated from soil microorganisms as well as investigating the antibiotic libraries of the Kitasato Institute for Life Sciences and BioFrontier Laboratories of

Kyowa Hakko Kogyo Co., Ltd. We have now discovered two compounds, KS-505a and alazopeptin, which exhibit moderate anti-trypanosomal characteristics. We report here the *in vitro* and *in vivo* antitrypanosomal activities and cytotoxicities of KS-505a and alazopeptin, compared with some commonly-used antitrypanosomal drugs. This is the first report of *in vitro* and *in vivo* antitrypanosomal activities of either KS-505a or alazopeptin.

14884. **Karioti, A., Skaltsa, H., Kaiser, M. & Tasdemir, D., 2009.** Trypanocidal, leishmanicidal and cytotoxic effects of anthecotulide-type linear sesquiterpene lactones from *Anthemis auriculata*. *Phytomedicine*. **In press, corrected proof.**

Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, 157 71 Athens, Greece.

Trypanosomiasis and leishmaniasis pose major public health threats for many countries, particularly those in sub-Saharan Africa and South America. In the present study, we evaluated the *in vitro* antiprotozoal activity of three irregular, linear sesquiterpene lactones recently isolated from Greek *Anthemis auriculata*, namely anthecotulide (1), 4-hydroxyanthecotulide (2) and 4-acetoxyanthecotulide (3). Trypomastigote forms of *Trypanosoma brucei rhodesiense* and *T. cruzi* as well as axenic amastigotes of *Leishmania donovani* were used for testing. The cytotoxic potential of the compounds was also assessed against mammalian (rat) skeletal myoblasts (L6 cells). All compounds showed potent trypanocidal and leishmanicidal activity. 4-Hydroxyanthecotulide (2) appeared to be the most active compound against all parasites, particularly towards *T. b. rhodesiense* (IC₅₀ 0.56µg/ml), whereas 4-acetoxyanthecotulide (3) was the least active. All three metabolites possessed toxicity on mammalian cells, which might limit their use as antiprotozoal agents.

14885. **Kaur, S., Shivange, A. V. & Roy, N., 2009.** Structural analysis of trypanosomal sirtuin: an insight for selective drug design. *MolecularDiversity*. **e Publication ahead of print, April 29.**

Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S Nagar, Mohali, Punjab, 160062, India.

The Trypanosomatidae family continues to create burdens on countries that are least equipped to bring new medicines to the clinic. For sickness caused by this family of parasites (African trypanosomiasis, Chagas disease, and leishmaniasis) no vaccines are available, and currently available drugs suffer from insufficient efficacy, excessive toxicity, and steady loss of effectiveness due to resistance. Availability of the genome sequence of pathogens of this family offers a unique avenue for the identification of novel common drug targets for all three pathogens. The sirtuin family of nicotinamide adenine dinucleotide (NAD)-dependent deacetylases is remarkably conserved throughout evolution from archaeobacteria to eukaryotes and plays an important role in trypanosomatidae biology and virulence. In order to gain insight for selective drug design, three-dimensional (3D) models of *L. major*, *L. infantum*, *T. brucei*, and *T. cruzi* sirtuin were constructed by homology modeling and compared with human sirtuin. The molecular electrostatic potentials and cavity depth analysis of these models suggest that the inhibitor binding catalytic domain has various minor structural differences in the active site of trypanosomal and human sirtuin, regardless of sequence

similarity. These studies have implications for designing effective strategies to identify inhibitors that can be developed as novel broad-spectrum antitrypanosomal drugs.

14886. **Kodama, H., Denso, Okazaki, F. & Ishida, S., 2008.** Protective effect of humus extract against *Trypanosoma brucei* infection in mice. *Journal of Veterinary Medical Science*, **70** (11): 1185-1190.

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Humic substances are formed during the decomposition of organic matter in humus, and are found in many natural environments in which organic materials and microorganisms are present. Oral administration of humus extract to mice successfully induced effective protection against experimental challenge by the two subspecies, *Trypanosoma brucei brucei* and *T. brucei gambiense*. Mortality was most reduced among mice who received a 3 percent humus extract for 21 days in drinking water *ad libitum*. Spleen cells from humus-administered mice exhibited significant non-specific cytotoxic activity against L1210 mouse leukemia target cells. Also, spleen cells produced significantly higher amounts of Interferon-gamma when stimulated *in vitro* with Concanavalin A than cells from normal controls. These results clearly show that administration to mice of humus extract induced effective resistance against *Trypanosoma* infection. Enhancement of the innate immune system may be involved in host defence against trypanosomiasis.

14887. **Link, A., Heidler, P., Kaiser, M. & Brun, R., 2009.** Synthesis of a series of N(6)-substituted adenosines with activity against trypanosomatid parasites. *European Journal of Medicinal Chemistry*. **In press, corrected proof.**

Institute of Pharmacy, Ernst-Moritz-Arndt-University, Friedrich-Ludwig-Jahn-Strasse 17, 17487 Greifswald, Germany.

The involvement of purine salvage in the accumulation of current trypanocidal drugs is important for the treatment of African sleeping sickness. The substrate specificity of essential nucleoside transporters is therefore of physiological and pharmacological interest. With the intention to contribute to the knowledge in the field, a series of 16 adenosine derivatives with substituents in N(6)-position were prepared in order to evaluate their potential to inhibit *Trypanosoma brucei* sp. *in vitro*. An unmodified ribose moiety was selected to conserve key molecular recognition motifs of the arsenal of integral membrane proteins expressed in large numbers on the protozoan plasma membrane. Two of the new compounds prepared using a polymer-assisted acylation protocol showed antitrypanosomal activities in the single digit μ molar concentration range.

14888. **Mdachi, R. E., Thuita, J. K., Kagira, J. M., Ngotho, J. M., Murilla, G. A., Ndung'u, J. M., Tidwell, R. R., Hall, J. E. & Brun, R., 2009.** Efficacy of the novel diamidine compound 2,5-Bis(4-amidinophenyl)-furan-bis-O-methylamidoxime (pafuramidine, DB289) against *Trypanosoma brucei rhodesiense* infection in vervet monkeys after oral administration. *Antimicrobial Agents and Chemotherapy*, **53** (3): 953-957.

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Owing to the lack of oral drugs for human African trypanosomiasis, patients have to be hospitalized for 10 to 30 days to facilitate treatment with parenterally administered medicines. The efficacy of a novel orally administered prodrug, 2,5-bis(4-amidinophenyl)-furan-bis-O-methylamidoxime (pafuramidine, DB289), was tested in the vervet monkey (*Chlorocebus [Cercopithecus] aethiops*) model of sleeping sickness. Five groups of three animals each were infected intravenously with 10^4 *Trypanosoma brucei rhodesiense* KETRI 2537 cells. On the seventh day post infection (p.i.) in an early-stage infection, animals in groups 1, 2, and 3 were treated orally with pafuramidine at dose rates of 1, 3, or 10 mg/kg of body weight, respectively, for five consecutive days. The animals in groups 4 and 5 were treated with 10 mg/kg for 10 consecutive days starting on the 14th day p.i. (group 4) or on the 28th day p.i. (group 5), when these animals were in the late stage of the disease. In the groups treated in the early stage, 10 mg/kg of pafuramidine completely cured all three monkeys, whereas lower doses of 3 mg/kg and 1 mg/kg cured only one of three and zero of three monkeys, respectively. Treatment of late-stage infections resulted in cure rates of one of three (group 4) and zero of three (group 5) monkeys. These studies demonstrated that pafuramidine was orally active in monkeys with early-stage *T. brucei rhodesiense* infections at dose rates above 3 mg/kg for 5 days. It was also evident that the drug attained only minimal efficacy against late-stage infections, indicating the limited ability of the molecule to cross the blood-brain barrier. This study has shown that oral diamidines have potential for the treatment of early-stage sleeping sickness.

14889. **Mogi, T., Ui, H., Shiomi, K., Omura, S., Miyoshi, H. & Kita, K., 2009.** Antibiotics LL-Z1272 identified as novel inhibitors discriminating bacterial and mitochondrial quinol oxidases. *Biochimica Biophysica Acta*, **1787** (2): 129-133.

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To counter antibiotic-resistant bacteria, we screened the Kitasato Institute for Life Sciences Chemical Library with bacterial quinol oxidase, which does not exist in the mitochondrial respiratory chain. We identified five prenylphenols, LL-Z1272beta, gamma, delta, varepsilon and zeta, as new inhibitors for the *Escherichia coli* cytochrome bd. We found that these compounds also inhibited the *E. coli* bo-type ubiquinol oxidase and trypanosome alternative oxidase, although these three oxidases are structurally unrelated. LL-Z1272beta and varepsilon (dechlorinated derivatives) were more active against cytochrome bd while LL-Z1272gamma, delta, and zeta (chlorinated derivatives) were potent inhibitors of cytochrome bo and trypanosome alternative oxidase. Thus prenylphenols are useful for the

selective inhibition of quinol oxidases and for understanding the molecular mechanisms of respiratory quinol oxidases as a probe for the quinol oxidation site. Since quinol oxidases are absent from mammalian mitochondria, LL-Z1272beta and delta, which are less toxic to human cells, could be used as lead compounds for development of novel chemotherapeutic agents against pathogenic bacteria and African trypanosomiasis.

14890. **Moreira, O. C., Rios, P. F., Esteves, F. F., Meyer-Fernandes, J. R. & Barrabin, H., 2009.** CrATP as a new inhibitor of ecto-ATPases of trypanosomatids. *Parasitology*, **136** (1): 35-44.

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Trypanosomatid protozoa include heteroxenic species some of them pathogenic for men, animals and plants. Parasite membrane contains ecto-enzymes whose active sites face the external medium rather than the cytoplasm. *Herpetomonas* sp. displayed a Mg²⁺-dependent ecto-ATPase activity, a Mg-independent ecto-ADPase and an ecto-phosphatase activity. Both, the ecto-ADPase and phosphatase activities were insensitive to CrATP (chromium(III) adenosine 5'-triphosphate complex). Ecto-ATPase activity was reversibly inhibited. At 2 µM ATP the apparent K_i was 4.7+/-1.0 µM but a fraction of about 40-50 percent was insensitive to CrATP. Remarkably, at low substrate concentration (0.2 mM) more than 90 percent of the ecto-ATPase was inhibited with K_i=0.33+/-1.0 µM. These parameter dependences are interpreted as the presence of 2 ecto-ATPases activities, one of them with high ATP apparent affinity and sensitivity to CrATP. DIDS (4,4 diisothiocyantostilbene 2,2' disulphonic acid), suramin and ADP were also effective as inhibitors. Only ADP presented no additive inhibition with CrATP. The pattern of partial inhibition by CrATP was also observed for the ecto-ATPase activities of *Leishmania amazonensis*, *Trypanosoma cruzi* and *Trypanosoma rangeli*. CrATP emerges as a new inhibitor of ecto-ATPases and as a tool for a better understanding of properties and role of ecto-ATPases in the biology of parasites.

14891. **Oluwafemi, A. J., Okanla, E. O., Camps, P., Munoz-Torrerob, D., Mackey, Z. B., Chiang, P. K., Seville, S. & Wright, C. W., 2009.** Evaluation of cryptolepine and huperzine derivatives as lead compounds towards new agents for the treatment of human African trypanosomiasis. *Natural Product Communications*, **4** (2): 193-198.

Department of Zoology, University of Ilorin, Ilorin, Nigeria.

The alkaloid cryptolepine (1) and eight synthetic analogues (2-8) were assessed for *in vitro* activities against *Trypanosoma brucei*. Four of the analogues were found to be highly potent with IC₅₀ values of less than 3 nM and three of these were assessed against *T. brucei* infection in rats. The most effective compound was 2, 7-dibromocryptolepine 7; a single oral dose of 20 mg/kg suppressed parasitaemia and increased the mean survival time to 13.6 days compared with 8.4 days for untreated controls. In addition, four huperzine derivatives (9-12) were shown to have *in vitro* anti-trypanosomal activities with IC₅₀ values ranging from 303-377 nM.

14892. **Patham, B., Duffy, J., Lane, A., Davis, R. C., Wipf, P., Fewell, S. W., Brodsky, J. L. & Mensa-Wilmot, K., 2009.** Post-translational import of protein into the endoplasmic reticulum of a trypanosome: an *in vitro* system for discovery of anti-trypanosomal chemical entities. *Biochemical Journal*, **419** (2): 507-517.

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HAT (human African trypanosomiasis), caused by the protozoan parasite *Trypanosoma brucei*, is an emerging disease for which new drugs are needed. Expression of plasma membrane proteins e.g. VSG (variant surface glycoprotein) is crucial for the establishment and maintenance of an infection by *T. brucei*. Transport of a majority of proteins to the plasma membrane involves their translocation into the ER (endoplasmic reticulum). Thus inhibition of protein import into the endoplasmic reticulum of *T. brucei* would be a logical target for discovery of lead compounds against trypanosomes. We have developed a *TbRM* (*T. brucei* microsome) system that imports VSG₁₁₇ post-translationally. Using this system, MAL3-101, equisetin and CJ-21 058 were discovered to be small molecule inhibitors of VSG₁₁₇ translocation into the ER. These agents also killed bloodstream *T. brucei in vitro*; the concentrations at which 50 percent of parasites were killed (IC₅₀) were 1.5 μ M (MAL3-101), 3.3 μ M (equisetin) and 7 μ M (CJ-21 058). Thus VSG₁₁₇ import into *TbRMs* is a rapid and novel assay to identify “new chemical entities” (e.g. MAL3-101, equisetin and CJ-21 058) for anti-trypanosome drug development.

14893. **Prasanna, S. & Doerksen, R. J., 2009.** Topological polar surface area: a useful descriptor in 2D-QSAR. *Current Medicinal Chemistry*, **16** (1): 21-41.

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Topological polar surface area (TPSA), which makes use of functional group contributions based on a large database of structures, is a convenient measure of the polar surface area that avoids the need to calculate ligand 3D structure or to decide which is the relevant biological conformation or conformations. We demonstrate the utility of TPSA in 2D-QSAR for 14 sets of diverse pharmacological activity data. Even though a large pool of reports showing the importance of the classic 2D descriptors such as calculated logP (ClogP) and calculated molar refractivity (CMR) exists in the 2D-QSAR literature, this is the first report to demonstrate the value of TPSA as a relevant descriptor applicable to a large, structurally and pharmacologically diverse set of classes of compounds. We also address the limitations of applicability of this descriptor for 2D-QSAR analysis. We observed a negative correlation of TPSA with activity data for anticancer alkaloids, MT1 and MT2 agonists, MAO-B and tumour necrosis factor-alpha inhibitors and a positive correlation with inhibitory activity data for telomerase, PDE-5, GSK-3, DNA-PK, aromatase, malaria, trypanosomatids and CB2 agonists.

14894. **Rubio, B. K., Tenney, K., Ang, K. H., Abdulla, M., Arkin, M., McKerrow, J. H. & Crews, P., 2009.** The marine sponge *Diacarnus bismarckensis* as a source of peroxiterpene inhibitors of *Trypanosoma brucei*, the causative agent of sleeping sickness. *Journal of Natural Products*, **72**(2):218-222.

Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California Santa Cruz, Santa Cruz, California, USA, Sandler Center for Basic Research in Parasitic Disease, University of California San Francisco, San Francisco, California, USA, and Small Molecule Discovery Center, University of California San Francisco, San Francisco, California, USA.

Human African trypanosomiasis, also known as African sleeping sickness, is a neglected tropical disease with inadequate therapeutic options. We have launched a collaborative new lead discovery venture using our repository of extracts and natural product compounds as input into our growth inhibition primary screen against *Trypanosoma brucei*. Careful evaluation of the spectral data of the natural products and derivatives allowed for the elucidation of the absolute configuration (using the modified Mosher's method) of two new peroxiterpenes: (+)-muquibilone B and (-)-ent-muquibilone. Five known compounds were also isolated: (+)-sigmosceptrin A, (+)-sigmosceptrin A methyl ester, (-)-sigmosceptrin B, (+)-epi-muquibillin A, and (-)-epi-nuapapu B methyl ester. The isolated peroxiterpenes demonstrated activities in the range IC(50) = 0.2-2 µg/mL.

14895. **Sanderson, L., Dogruel, M., Rodgers, J., De Koning, H. & Thomas, S. A., 2009.** Pentamidine movement across the murine blood-brain and blood-CSF barriers; effect of trypanosome infection, combination therapy, P-glycoprotein and MRP. *Journal of Pharmacology and Experimental Therapeutics*. **Fast Forward Publication, March 4.**

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During the first stage of human African trypanosomiasis (HAT), *T. b. gambiense* are found mainly in the blood and pentamidine treatment is used. Pentamidine is predominately ineffective once the parasites have invaded the CNS. This lack of efficacy is thought to be due to the inability of pentamidine to cross the blood-brain barrier, although this has never been directly explored. This study addresses this using brain perfusion in healthy mice, P-glycoprotein-deficient mice and in a murine model of HAT (*T. b. brucei*). The influence of additional anti-trypanosomal drugs on pentamidine delivery to the CNS was also investigated. Results revealed that ³H pentamidine can cross the blood-brain barrier, although a proportion was retained by the capillary endothelium and failed to reach the healthy or trypanosome-infected brain (up to day 21 p.i.). The CNS distribution of pentamidine was increased in the final (possibly terminal) stage of trypanosome infection partly due to loss of barrier integrity (day 28-35 p.i.) as measured by ¹⁴C sucrose and ³H suramin. Furthermore, pentamidine distribution to the CNS involved influx and efflux (via P-glycoprotein and multi-drug resistance associated protein (MRP)) transporters and was affected by the other anti-trypanosomal agents, suramin, melarsoprol and nifurtimox, but not eflornithine. These interactions could contribute to side effects or lead to the development of parasite resistance to the drugs. Thus great care must be taken when designing drug combinations containing

pentamidine or other diamidine analogues. However, co-administration of P-glycoprotein and/or MRP inhibitors with pentamidine, or other diamidines, might provide a means of improving efficacy against CNS stage HAT.

14896. **Smith, T. K., Young, B. L., Denton, H., Hughes, D. L. & Wagner, G. K., 2009.** First small molecular inhibitors of *T. brucei* dolicholphosphate mannose synthase (DPMS), a validated drug target in African sleeping sickness. *Bioorganic and Medicinal Chemistry Letters*, **19** (6): 1749-1752.

Centre for Biomolecular Sciences, The North Haugh, The University, St. Andrews, Scotland, UK.

Drug-like molecules with activity against *Trypanosoma brucei* are urgently required as potential therapeutics for the treatment of African sleeping sickness. Starting from known inhibitors of other glycosyltransferases, we have developed the first small molecular inhibitors of dolicholphosphate mannose synthase (DPMS), a mannosyltransferase critically involved in glycoconjugate biosynthesis in *T. brucei*. We show that these DPMS inhibitors prevent the biosynthesis of glycosylphosphatidylinositol (GPI) anchors, and possess trypanocidal activity against live trypanosomes.

14897. **Steverding, D. & Wang, X., 2009.** Evaluation of anti-sleeping-sickness drugs and topoisomerase inhibitors in combination on *Trypanosoma brucei*. *Journal of Antimicrobial Chemotherapy*, **63**:1293-1295.

BioMedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, UK.

No abstract available.

14898. **Toriiizuka, Y., Kinoshita, E., Kogure, N., Kitajima, M., Ishiyama, A., Otaguro, K., Yamada, H., Omura, S. & Takayama, H., 2008.** New lycorine-type alkaloid from *Lycoris traubii* and evaluation of antitrypanosomal and antimalarial activities of lycorine derivatives. *Bioorganic and Medicinal Chemistry*, **16** (24): 10182-10189.

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A new lycorine derivative LT1 (4) was isolated from the aerial part and bulbs of *Lycoris traubii* Hayward (*Amaryllidaceae*). Its structure including absolute configuration was established by spectroscopic analysis and semi-synthesis to be 1-O-(3'S)-hydroxybutanoyllycorine. Some lycorine ester derivatives including LT1 were examined for their inhibitory activity against *Trypanosoma brucei brucei*, the parasite associated with sleeping sickness, and against *Plasmodium falciparum*, the causative agent of malaria. Among them, 2-O-acetyllycorine (6) showed the most potent activity against parasitic *T. b. brucei*, and LT1 (4), 1-O-(3'R)-hydroxybutanoyllycorine (8), 1,2-di-O-butanoyllycorine (11), and 1-O-propanoyllycorine (12) showed significant activity against *P. falciparum* in an *in vitro* experiment.

14899. **Vanhamme, L., 2008.** Trypanosome RNA polymerases and transcription factors: sensible trypanocidal drug targets? *Current Drug Targets*, **9** (11): 979-996.

Laboratoire de Parasitologie Moléculaire, Institut de Biologie et Médecine Moléculaire (IBMM), Université Libre de Bruxelles (ULB), 12 rue des Professeurs Jeener et Brachet, 6041 Gosselies, Belgium.
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Trypanosomes and *Leishmania* are the agents of several important parasitic diseases threatening hundreds of million human beings worldwide. As they diverged early in evolution, they display original molecular characteristics. These peculiarities are each defining putative specific targets for anti-parasitic drugs. Transcription displays its lot of unique characteristics in trypanosomes and will be taken as an example to uncover these targets. Unique features of transcription in trypanosomes include constitutive and polycistronic transcription by RNA polymerase II as well as transcription of protein-coding genes by RNA polymerase I. It is becoming clear that these unique mechanisms are performed by dedicated molecular players. The first of them have been recently characterized. They are reviewed and their suitability as drug targets is commented.

14900. **Weis, R. & Seebacher, W., 2009.** New bicyclic amines: synthesis and SARs of their action against the causative organisms of malaria and sleeping sickness. *Current Medicinal Chemistry*, **16** (11): 1426-1441.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, A-8010 Graz, Austria. [we.seebacher@uni-graz.at].

Diaryl-substituted bicyclic amines are a scarcely investigated class of compounds. Only few of them are described and their biological activities are reported poorly. During our work in the field of heterocyclic chemistry, we found that 4-dialkylaminobicyclo[2.2.2]octan-2-ones and -ols show antiprotozoal properties against *Plasmodium falciparum* K(1) and *Trypanosoma brucei rhodesiense*, the causative organisms of tropical malaria and of human African trypanosomiasis. Therefore, we synthesized over 200 derivatives in order to investigate their anti-trypanosomal and anti-plasmodial activities as well as their cytotoxicity using *in vitro* microplate assays. Even if the target and the mechanism of action of these compounds are still unknown, we can at least provide several structure-activity relationships for this interesting class of compounds. Moreover, we achieved a distinct improvement of their antiplasmodial and antitrypanosomal properties.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

14901. **Delespaux, V., Dinka, H., Masumu, J., Van den Bossche, P. & Geerts, S., 2008.** Five-fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven-year period in Eastern Zambia. *Drug Resistance Updates*, **11** (6): 205-209.

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Two groups of *Trypanosoma congolense* isolates collected from cattle in 1996 (n=39) and 2003 (n=38) in the Eastern Province of Zambia were analyzed by BclI-PCR-RFLP to assess the evolution of diminazene aceturate (DA) resistance over a period of seven years. The results show a significant increase of DA resistance in this relatively short period of time. In 1996, among the 39 isolates, 61.5 percent were found sensitive, 12.8 percent resistant and 25.7 percent had a mixed BclI-PCR-RFLP profile. In 2003, among the 38 isolates, 10.5 percent were found sensitive, 63.2 percent were resistant and 26.3 percent showed a mixed BclI-PCR-RFLP profile. *In vivo* tests in mice showed that isolates with a sensitive or mixed RFLP profile were sensitive to DA whereas isolates with a resistant RFLP profile were resistant. Since there are no indications that the drug pressure has increased between 1996 and 2003, it is suggested that genetic exchange of resistance genes might explain the increased frequency of resistance to DA.

14902. **Hamilton, P. B., Adams, E. R., Njiokou, F., Gibson, W. C., Cuny, G. & Herder, S., 2009.** Phylogenetic analysis reveals the presence of the *Trypanosoma cruzi* clade in African terrestrial mammals. *Infection, Genetics and Evolution*, **9** (1): 81-86.

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Despite the impact of some trypanosome species on human and livestock health, the full diversity of trypanosomes in Africa is poorly understood. A recent study examined the prevalence of trypanosomes among a wide variety of wild vertebrates in Cameroon using species-specific PCR tests, but six trypanosome isolates remained unidentified. Here they have been re-examined using fluorescent fragment length barcoding (FFLB) and phylogenetic analysis of glycosomal glyceraldehyde phosphate dehydrogenase gGAPDH and 18S ribosomal RNA (rRNA) genes. Isolates from a monkey (*Cercopithecus nictitans*) and a palm civet (*Nandinia binotata*) belonged to the *Trypanosoma cruzi* clade, known previously only from New World and Australian terrestrial mammals, and bats from Africa, Europe and South America. Of the four other isolates, three from antelope were identified as *Trypanosoma theileri*, and one from a crocodile as *T. grayi*. This is the first report of trypanosomes of the *T. cruzi* clade in African terrestrial mammals and expands the clade's

known global distribution in terrestrial mammals. Previously it has been hypothesized that African and New World trypanosomes diverged after continental separation, dating the divergence to around 100 million years ago. The new evidence instead suggests that intercontinental transfer occurred well after this, possibly via bats or rodents, allowing these trypanosomes to establish and evolve in African terrestrial mammals, and questioning the validity of calibrating trypanosome molecular trees using continental separation.

14903. **Madeira, M. F., Sousa, M. A., Barros, J. H., Figueiredo, F. B., Fagundes, A., Schubach, A., CC, D. E. P., Faissal, B. N., Fonseca, T. S., Thoma, H. K. & Marzochi, M. C., 2009.** *Trypanosoma caninum* n. sp. (Protozoa: Kinetoplastida) isolated from intact skin of a domestic dog (*Canis familiaris*) captured in Rio de Janeiro, Brazil. *Parasitology*, **136** (4): 411-423.

Laboratório de Vigilância em Leishmanioses, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil. [fatima.madeira@ipecc.fiocruz.br].

An unknown *Trypanosoma* species was isolated from an axenic culture of intact skin from a domestic dog captured in Rio de Janeiro, Brazil, which was co-infected with *Leishmania (Viannia) braziliensis*. Giemsa-stained smears of cultures grown in different media revealed the presence of epimastigotes, trypomastigotes, spheromastigotes, transitional stages, and dividing forms (epimastigotes or spheromastigotes). The highest frequency of trypomastigotes was observed in RPMI (15.2 percent) and DMEM (9.2 percent) media containing 5 percent FCS, with a mean length of these forms of 43.0 and 36.0 µm, respectively. Molecular analysis by sequential application of PCR assays indicated that this trypanosome differs from *Trypanosoma cruzi* and *T. rangeli* when specific primers were applied. On the other hand, a PCR strategy targeted to the D7 domain of 24Sα rDNA, using primers D75/D76, amplified products of about 250 bp in that isolate (stock A-27), different from the amplification products obtained with *T. cruzi* and *T. rangeli*. This organism differs from *T. cruzi* mainly by the size of its trypomastigote forms and kinetoplasts and the absence of infectivity for macrophages and triatomine bugs. It is also morphologically distinct from salivarian trypanosomes reported in Brazil. Isoenzyme analysis at 8 loci demonstrated a very peculiar banding pattern clearly distinct from those of *T. rangeli* and *T. cruzi*. We conclude that this isolate is a new *Trypanosoma* species. The name *T. caninum* is suggested.

14904. **Masumu, J., Geysen, D. & Bossche, P. V., 2009.** Endemic type of animal trypanosomiasis is not associated with lower genotype variability of *Trypanosoma congolense* isolates circulating in livestock. *Research in Veterinary Science*. **In press, corrected proof.**

University of Pretoria, Department of Veterinary Tropical Diseases, Onderstepoort, Pretoria, Gauteng 0110, South Africa.

In order to verify whether the low impact on livestock production in endemic areas is related to a low number of trypanosome strains circulating in livestock, 37 *Trypanosoma congolense* isolates collected from cattle in 11 sites in an endemic trypanosomiasis area in Eastern Zambia were characterised for genotype variability using a modified amplified

fragment length polymorphism technique (AFLP). Isolates were further cloned to evaluate the occurrence of mixed infections in individuals. The results obtained revealed a high genotype diversity (94.6 percent) among these isolates. Apart from one site, all isolates gave different AFLP profiles in each of the sites. When clones were compared, three (8 percent) of the 37 isolates had mixed infections. These results indicate the circulation of a high number of strains in this trypanosomiasis endemic area despite the low impact the disease has on livestock production.

14905. **Poinar Jr, G., 2008.** *Leptoconops nosopheris* sp. n. (Diptera: *Ceratopogonidae*) and *Paleotrypanosoma burmanicus* gen. n., sp. n. (Kinetoplastida: *Trypanosomatidae*), a biting midge-trypanosome vector association from the Early Cretaceous. *Memórias do Instituto Oswaldo Cruz*, **103** (5): 468-471.

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Leptoconops nosopheris sp. n. is described from a blood-filled female biting midge in Early Cretaceous Burmese amber. The new species is characterized by a very elongate terminal flagellomere, elongated cerci, and an indistinct spur on the metatibia. This biting midge contained digenetic trypanosomes (Kinetoplastida: *Trypanosomatidae*) in its alimentary tract and salivary glands. These trypanosomes are described as *Paleotrypanosoma burmanicus* gen. n., sp. n., which represents the first fossil record of a *Trypanosoma* generic lineage.

14906. **Sato, H., Takano, A., Kawabata, H., Une, Y., Watanabe, H. & Mukhtar, M. M., 2009.** *Trypanosoma* cf. *varani* in an imported ball python (*Python reginus*) from Ghana. *Journal of Parasitology*. e Publication ahead of print, January 12.

Yamaguchi University and National Institute of Infectious Diseases, Japan and Azabu University and University of Khartoum, Sudan.

Peripheral blood from a ball python (*Python reginus*) imported from Ghana was cultured in Barbour-Stoenner-Kelly (BSK) medium for *Borrelia* sp. isolation, resulting in the prominent appearance of free, and clusters of, trypanosomes in a variety of morphological forms. The molecular phylogenetic characterization of these cultured trypanosomes using the small subunit rDNA indicated that this python was infected with a species closely related to *Trypanosoma varani* Wenyon, 1908, originally described in the Nile monitor lizard (*Varanus niloticus*) from Sudan. Furthermore, nucleotide sequences of glycosomal glyceraldehyde-3-phosphate dehydrogenase gene of both isolates showed few differences. Giemsa-stained blood smears prepared from the infected python 8 months after the initial observation of trypanosomes in haemoculture contained trypomastigotes with a broad body and a short, free flagellum, which most closely resembled to the original description of *T. varani*, or *T. voltariae* Macfie, 1919 recorded in a black-necked spitting cobra (*Naja nigricollis*) from Ghana. It is highly possible that lizards and snakes could share naturally an identical trypanosome species. Alternatively, lizards and snakes in the same region might have closely-related, but distinct, *Trypanosoma* species as a result of sympatric speciation. From multiple viewpoints, including molecular phylogenetic analyses, reappraisal of trypanosome species

from a wide range of reptiles in Africa is needed to clarify the relationship of recorded species or to unmask unrecorded species.

14907. **Yurchenko, V. Y., Lukes, J., Jirku, M. & Maslov, D. A., 2009.** Selective recovery of the cultivation-prone components from mixed trypanosomatid infections: a case of several novel species isolated from *Neotropical heteroptera*. *International Journal of Systemic Evolution and Microbiology*, **59** (4): 893-909.

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Mixed trypanosomatid infections (a simultaneous presence of two or more parasites in the same host) have long been suspected to represent an obstacle for recovering cultures that would faithfully represent original species descriptions. However, without the means to directly compare the parasites in the host and in culture, this would remain just a possibility. Here we have used PCR-based genotyping of spliced leader RNA gene repeats to analyse several novel species of insect trypanosomatids isolated from heteropteran hosts and to compare them with the parasites that had been detected in the gut smears of the same hosts. We have found that, whereas the original infections were dominated by some blastocrithidia-like parasites, most of the respective axenic cultures contained novel species of *Crithidia* and *Leptomonas*. Therefore, we concluded that, in each case, this replacement was caused by differences in cultivation properties between the original predominant blastocrithidia and the less fastidious parasite that was later recovered in culture. The properties of the new organisms, including their morphology and ultrastructure, as well as their phylogenetic affinities within the family, were investigated and used to describe five novel species.

(c) LIFE CYCLE, MORPHOLOGY, BIOCHEMISTRY AND MOLECULAR STUDIES

14908. **Adhiambo, C., Blisnick, T., Toutirais, G., Delannoy, E. & Bastin, P., 2009.** A novel function for the atypical small G protein Rab-like 5 in the assembly of the trypanosome flagellum. *Journal of Cell Science*, **122** (6): 834-841.

Trypanosome Cell Biology Unit, Pasteur Institute and CNRS, Paris, France.

The atypical small G protein Rab-like 5 has been shown to traffic in sensory cilia of *Caenorhabditis elegans*, where it participates in signalling processes but not in cilia construction. In this report, we demonstrate that RABL5 co-localises with intraflagellar transport (IFT) proteins at the basal body and in the flagellum matrix of the protist *Trypanosoma brucei*. RABL5 fused to GFP exhibits anterograde movement in the flagellum of live trypanosomes, suggesting it could be associated with IFT. Accordingly, RABL5 accumulates in the short flagella of the retrograde IFT140(RNAi) mutant and is restricted to the basal body region in the IFT88(RNAi) anterograde mutant, a behaviour that is identical to other IFT proteins. Strikingly, RNAi silencing reveals an essential role for RABL5 in trypanosome flagellum construction. RNAi knock-down produces a phenotype similar to inactivation of retrograde IFT with formation of short flagella that are filled with a high amount of IFT proteins. These data reveal for the first time a functional difference for a

conserved flagellar matrix protein between two different ciliated species and raise questions related to cilia diversity.

14909. **Allen, J. W., Ferguson, S. J. & Ginger, M. L., 2008.** Distinctive biochemistry in the trypanosome mitochondrial intermembrane space suggests a model for stepwise evolution of the MIA pathway for import of cysteine-rich proteins. *FEBS Letters*, **582** (19): 2817-2825.

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Mia 40-dependent disulphide bond exchange is used by animals, yeast, and probably plants for import of small, cysteine-rich proteins into the mitochondrial intermembrane space (IMS). During import, electrons are transferred from the imported substrate to Mia40 then, via the sulphhydryl oxidase Erv1, into the respiratory chain. Curiously, however, there are protozoa which contain substrates for Mia 40-dependent import, but lack Mia 40. There are also organisms where Erv1 is present in the absence of respiratory chain components. In accommodating these and other relevant observations pertaining to mitochondrial cell biology, we hypothesise that the ancestral IMS import pathway for disulphide-bonded proteins required only Erv1 (but not Mia 40) and identify parasites in which O₂ is the likely physiological oxidant for Erv1.

14910. **Archer, S., Queiroz, R., Stewart, M. & Clayton, C., 2008.** Trypanosomes as a model to investigate mRNA decay pathways. *Methods in Enzymology*, **448**: 359-377.

Zentrum für Molekulare Biologie der Universität Heidelberg, Heidelberg, Germany.

In trypanosomes, individual mRNAs arise by the processing of primary polycistronic transcripts. Consequently, mRNA degradation rates are critical determinants of mRNA abundance. In this chapter, we summarize the various options for genetic manipulation in trypanosomes with the goal of analyzing mRNA stability, including RNA interference. We describe a method for measuring the half-lives of trypanosome mRNAs, including those that are very unstable, and also the isolation of tagged protein-RNA complexes by IgG affinity chromatography. Last, we detail our current methods for RNA analysis with microarrays.

14911. **Barquilla, A., Crespo, J. L. & Navarro, M., 2008.** Rapamycin inhibits trypanosome cell growth by preventing TOR complex 2 formation. *Proceedings of the National Academy of Sciences U S A*, **105** (38): 14579-14584.

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Target of rapamycin (TOR) kinases control cell growth through two functionally distinct multiprotein complexes. TOR complex 1 (TORC1) controls temporal cell growth and is sensitive to rapamycin, whereas TOR complex 2 (TORC2) is rapamycin resistant and

regulates spatial cell growth. Here, we identified two TOR orthologues, *TbTOR1* and *TbTOR2*, in the protozoan parasite *Trypanosoma brucei*, as well as orthologues of the well-known TORC1 and TORC2 partners, KOG1/raptor and AVO3/ricin. *TbTOR* proteins differ in their functions, subcellular localization, and rapamycin sensitivity. *TbTOR1* controls cell growth by regulating cell cycle, nucleolus structure, and protein synthesis, whereas *TbTOR2* coordinates cell polarization and cytokinesis. Rapamycin treatment of bloodstream trypanosomes resulted in a pronounced reduction of cell proliferation, with an EC(50) of 152 nM. Unique for a eukaryote, we observed that rapamycin acted exclusively by preventing TORC2 formation, with no effect on TORC1. Our findings on TOR signalling in this protozoan, which is located in a distal position in the eukaryotic cell lineage, highlight the clinical possibilities of rapamycin derivatives and provide valuable insights into understanding rapamycin-mediated inhibition of TORC2.

14912. **Barquilla, A. & Navarro, M., 2009.** Trypanosome TOR as a major regulator of cell growth and autophagy. *Autophagy*, **5** (2): 256-258.

Instituto de Parasitología y Biomedicina Lopez-Neyra, Consejo Superior de Investigaciones Científicas, CSIC, (Spanish National Research Council), Granada, Spain.

Trypanosomatid protozoa parasites are responsible for tropical diseases, and undergo complex life cycles involving developmental forms adapted to insect vectors and vertebrate hosts. During their life cycle these parasites proceed through different forms in response to dramatic environmental changes and/or developmentally regulated programs. Successful progression of the parasite through its life cycle is highly dependent on the capacity of adaptation to distinct stresses involving processes such as autophagy. In eukaryotes, target of rapamycin (TOR) protein kinases act as a sensor, which integrates the nutritional and energetic status, adjusting cell metabolism and growth. Compromising cell viability in yeast and mammals leads to a reduction of TOR function, triggering processes aimed to overcome unfavourable conditions. This is partly achieved by TOR-mediated regulation of protein synthesis and recycling of cellular components by autophagy. In the last few years, autophagy has been described during developmental differentiation processes in *Trypanosomatidae*. However, no link between TOR signalling, autophagy, and differentiation has been described so far. This addendum is a commentary to the work published by our group, in which we discuss the possible role of TOR kinases, as a controller of cell growth and autophagy, in the regulation of differentiation processes during Trypanosomatids life cycles.

14913. **Barquilla, A. & Navarro, M., 2009.** Trypanosome TOR complex 2 functions in cytokinesis. *Cell Cycle*, **8** (5): 697-699.

Instituto de Parasitología y Biomedicina Lopez-Neyra, Consejo Superior de Investigaciones Científicas, CSIC, (Spanish National Research Council), Avda. del Conocimiento s/n, Granada, Spain.

TOR (target of rapamycin) is a kinase of the phosphatidylinositol kinase-related kinase (PIKK) family that controls cell growth in eukaryotes in response to nutrients, energy conditions and growth factors. We have recently identified two trypanosome TOR

orthologues, named *TbTOR1* and *TbTOR2*, and two other proteins with significant homology to yeast or mammalian TORs, named *TbTOR-like 1* and *TbTOR-like 2*. *TbTOR1* depletion results in arrest of bloodstream trypanosomes in G(1), concomitant to protein synthesis inhibition; however, *TbTOR2* depletion leads to dramatic morphological defects in cell polarization, endocytosis and cytokinesis. Rapamycin inhibits *T. brucei* cell growth by prevention of TORC2 complex formation, without any effect on TORC1 contrary to what generally occurs in other eukaryotes. Based on the unique features of *T. brucei* and its distal position in the eukaryotic cell lineage, we describe our views on the function of the TOR protein as a major regulator of cell growth and cytokinesis and discuss a possible role in the developmental differentiation processes.

14914. **Bercovich, N., Levin, M. J. & Vazquez, M. P., 2009.** The FIP-1 like polyadenylation factor in trypanosomes and the structural basis for its interaction with CPSF30. *Biochemica and Biophysica Research Communications*, **380** (4): 850-855.

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In trypanosomes transcription is polycistronic and individual mRNAs are generated by a trans-splicing/polyadenylation coupled reaction. We identified a divergent trypanosome FIP1-like, a factor required for mRNA 3' end formation from yeasts to human. Here we show that it is a nuclear protein with a dotted distribution essential for trypanosome viability. A strong interaction was found between *TcFIP1*-like and *TcCPSF30*, a component of the polyadenylation complex. We determined the specific amino acids in each protein involved in the interaction. Significant differences were found between the trypanosome interaction surface and its human counterpart. Although CPSF30/FIP1 interaction is known in other organisms, this is the first report mapping the interaction surface at the amino acid level.

14915. **Casanova, M., Crobu, L., Blaineau, C., Bourgeois, N., Bastien, P. & Pages, M., 2009.** Microtubule-severing proteins are involved in flagellar length control and mitosis in Trypanosomatids. *Molecular Microbiology*, **71** (6): 1353-1370.

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Microtubules are key players in the biology of Trypanosomatid parasites, not only as classical components of the mitotic spindle, microtubule-organizing centres and flagellum but also as the essential constituent of the cytoskeleton. Their length dynamics are regulated by, among others, microtubule-severing proteins. Four and six genes encoding microtubule-severing proteins can be found bioinformatically in the *Leishmania major* and *Trypanosoma brucei* genome respectively. We investigated all these proteins in these organisms, which include the katanin, katanin-like, spastin and fidgetin, and looked at their subcellular localization as well as their putative function by examining "loss-of-function" phenotypes. The katanin-like KAT60b was found implicated in flagellar length reduction, but not in its size increase, while the katanin p80 subunit appeared clearly involved in cytokinesis. Fidgetin and spastin homologues were both localized in the nucleus: the first as a discrete and variable number of dots during most of the cell cycle, redistributing to the spindle and midbody during

mitosis; the second concentrated as $< \text{ or } = 5$ perinucleolar punctuations, similar to the electron-dense plaques identified in *T. brucei*, which were assimilated to kinetochores. This first study of microtubule-severing proteins in 'divergent' eukaryotes gives further insight into the multiple functions of these proteins identified in the hitherto studied models.

14916. **Charriere, F., O'Donoghue, P., Helgadottir, S., Marechal-Drouard, L., Cristodero, M., Horn, E. K., Soll, D. & Schneider, A., 2009.** Dual targeting of a tRNA ASP requires two different aspartyl-tRNA synthetases in *Trypanosoma brucei*. *Journal of Biological Chemistry*. **e Publication ahead of print, April 22.**

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The mitochondrion of the parasitic protozoan *Trypanosoma brucei* does not encode any tRNAs. This deficiency is compensated for by partial import of nearly all of its cytosolic tRNAs. Most trypanosomal aminoacyl-tRNA synthetases are encoded by single copy genes suggesting the use of the same enzyme in the cytosol and in the mitochondrion. However, the *T. brucei* genome encodes two distinct genes for eukaryotic aspartyl-tRNA synthetase (AspRS) even though the cell has a single tRNA(Asp) isoacceptor only. Phylogenetic analysis showed that the two *T. brucei* AspRSs evolved from a duplication early in kinetoplastid evolution and also revealed that 8 other major duplications of AspRS occurred in the eukaryotic domain. RNAi analysis established that both *Tb*-AspRS1 and *Tb*-AspRS2 are essential for growth and required for cytosolic and mitochondrial Asp-tRNA(Asp) formation, respectively. *In vitro* charging assays demonstrated that the mitochondrial *Tb*-AspRS2 aminoacylates both cytosolic and mitochondrial tRNA(Asp), whereas the cytosolic *Tb*-AspRS1 selectively recognizes cytosolic but not mitochondrial tRNA(Asp). This indicates that cytosolic and mitochondrial tRNA(Asp), even though derived from the same nuclear gene, are physically different most likely due to a mitochondria-specific nucleotide modification. Mitochondrial *Tb*-AspRS2 defines a novel group of eukaryotic AspRSs with an extended substrate specificity that is restricted to trypanosomatids and therefore may be exploited as a novel drug target.

14917. **Cliffe, L. J., Kieft, R., Southern, T., Birkeland, S. R., Marshall, M., Sweeney, K. & Sabatini, R., 2009.** JBP1 and JBP2 are two distinct thymidine hydroxylases involved in J biosynthesis in genomic DNA of African trypanosomes. *Nucleic Acids Research*, **37** (5): 1452-1462.

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Genomic DNA of African trypanosomes contains a hypermodified thymidine residue termed base J (beta-d-glucosyl-HOMedU). This modified base is localized primarily to repetitive DNA, namely the telomeres, and is implicated in the regulation of antigenic variation. The base is synthesized in a two-step pathway. Initially, a thymidine residue in DNA is hydroxylated by a thymidine hydroxylase (TH). This intermediate (HOMedU) is then glucosylated to form base J. Two proteins involved in J synthesis, JBP1 (J binding protein 1) and JBP2, contain a putative TH domain related to the family of Fe(2+)/2-oxoglutarate-dependent hydroxylases. We have previously shown that mutations in the TH domain of JBP1 kill its ability to stimulate J synthesis. Here we show that mutation of key residues in

the TH domain of JBP2 ablate its ability to induce *de novo* J synthesis. While the individual JBP1 null and JBP2 null trypanosomes have reduced J levels, the deletion of both JBP1 and JBP2 generates a cell line that completely lacks base J but still contains glucosyl-transferase activity. Reintroduction of JBP2 in the J-null trypanosome stimulates HOMedU formation and site-specific synthesis of base J. We conclude that JBP2 and JBP1 are the TH enzymes involved in J biosynthesis.

14918. **Cordeiro, A. T., Thiemann, O. H. & Michels, P. A., 2009.** Inhibition of *Trypanosoma brucei* glucose-6-phosphate dehydrogenase by human steroids and their effects on the viability of cultured parasites. *Bioorganic and Medicinal Chemistry*, **17** (6): 2483-2489.

Centro de Biologia Molecular Estrutural, Instituto de Física de São Carlos, Universidade de São Paulo, Av. Trabalhador São Carlense 400, São Carlos, Brazil. [arturcor@gmail.com].

Dehydroepiandrosterone (DHEA) is known as an intermediate in the synthesis of mammalian steroids and a potent uncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH), but not of the enzyme from plants and lower eukaryotes. G6PDH catalyzes the first step of the pentose-phosphate pathway supplying cells with ribose 5-phosphate, a precursor of nucleic acid synthesis, and NADPH for biosynthetic processes and protection against oxidative stress. In this paper we demonstrate that also G6PDH of the protozoan parasite *Trypanosoma brucei* is uncompetitively inhibited by DHEA and epiandrosterone (EA), with K(i) values in the lower μM range. A viability assay confirmed the toxic effect of both steroids on cultured *T. brucei* bloodstream form cells. Additionally, RNAi mediated reduction of the G6PDH level in *T. brucei* bloodstream forms validated this enzyme as a drug target against human African trypanosomiasis. Together these findings show that inhibition of G6PDH by DHEA derivatives may lead to the development of a new class of anti-trypanosomatid compounds.

14919. **Denninger, V., Koopmann, R., Muhammad, K., Barth, T., Bassarak, B., Schonfeld, C., Kilunga, B. K. & Duszenko, M., 2008.** Kinetoplastida: model organisms for simple autophagic pathways? *Methods in Enzymology*, **451**: 373-408.

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Phylogenetic analyses based on defined proteins or different RNA species have revealed that the order Kinetoplastida belongs to the early-branching eukaryotes and may thus contain organisms in which complex cellular events are easier to analyze. This view was further supported by results from a bioinformatic survey that suggested that nearly half of the autophagy-related proteins existent in yeast are missing in trypanosomatids. On the other hand, these organisms have evolved a highly sophisticated machinery to escape from the different host immune-response strategies and have learned to cope with extremely variable environmental conditions by morphological and functional reorganization of the cell. For both the stress response and the differentiation processes, autophagy seems to be an indispensable prerequisite. So far autophagy has not been systematically investigated in

trypanosomatids. Here we present technical information on how to handle the different parasites belonging to this order and give an overview of the current status of autophagy research in these organisms.

14920. **de Souza, W., Attias, M. & Rodrigues, J. C., 2009.** Particularities of mitochondrial structure in parasitic protozoa (Apicomplexa and Kinetoplastida). *International Journal of Biochemistry and Cell Biology*. **In press, corrected proof.**

Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, CCS-Bloco G, Ilha do Fundão, 21941-902, Rio de Janeiro-RJ, Brazil; Diretoria de Programas, Instituto Nacional de Metrologia e Qualidade Industrial-INMETRO, Rio de Janeiro, Brazil..

Without mitochondria, eukaryotic cells would depend entirely on anaerobic glycolysis for ATP generation. This also holds true for Protists, both free-living and parasitic. Parasitic Protists include agents of human and animal diseases that have a huge impact on world populations. In the phylum Apicomplexa, several species of *Plasmodium* cause malaria, whereas *Toxoplasma gondii* is a cosmopolite parasite found on all continents. Flagellates of the order Kinetoplastida include the genera *Leishmania* and *Trypanosoma* causative agents of human leishmaniasis and (depending on the species) African trypanosomiasis and Chagas disease. Although clearly distinct in many aspects, the members of these two groups bear a single and usually well developed mitochondrion. The single mitochondrion of Apicomplexa has a dense matrix and many cristae with a circular profile. The organelle is even more peculiar in the order kinetoplastida, exhibiting a condensed network of DNA at a specific position, always close to the flagellar basal body. This arrangement is known as Kinetoplast and the name of the Order is derived from it. Kinetoplastids also bear glycosomes, peroxisomes that concentrate enzymes of the glycolytic cycle. Mitochondrial volume and activity are maximum when glycosomal activity is low and *vice versa*. In both Apicomplexa and Trypanosomatids, mitochondria show particular features that are absent in other eukaryotic organisms. These peculiar features make them an attractive target for therapeutic drugs for the diseases they cause.

14921. **Duclert-Savatier, N., Poggi, L., Miclet, E., Lopes, P., Ouazzani, J., Chevalier, N., Nilges, M., Delarue, M. & Stoven, V., 2009.** Insights into the enzymatic mechanism of 6-phosphogluconolactonase from *Trypanosoma brucei* using structural data and molecular dynamics simulation. *Journal of Molecular Biology*, **366** (3): 868-881.

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Trypanosoma brucei is the causative agent of African sleeping sickness. Current work for the development of new drugs against this pathology includes evaluation of enzymes of the pentose phosphate pathway (PPP), which first requires a clear understanding of their function and mechanism of action. In this context, we focused on *T. brucei* 6-phosphogluconolactonase (*Tb6PGL*), which converts delta-6-phosphogluconolactone into 6-phosphogluconic acid in the second step of the PPP. We have determined the crystal structure

of Tb6PGL is complex with two ligands, 6-phosphogluconic acid and citrate, at 2.2 Å and 2.0 Å resolution, respectively. We have performed molecular dynamics (MD) simulations on Tb6PGL in its empty form and complexed with delta-6-phosphogluconolactone, its natural ligand. Analysis of the structural data and MD simulations allowed us to propose a detailed enzymatic mechanism for 6PGL enzymes.

14922. **Dutra, P. M., Dias, F. A., Santos, M. A., Rodrigues, C. O., Romeiro, A., Attias, M., De Souza, W., Lopes, A. H. & Meyer-Fernandes, J. R., 2001.** Secreted phosphatase activities in trypanosomatid parasites of plants modulated by platelet-activating factor. *Phytopathology*, **91** (4): 408-414.

Instituto de Microbiologia Professor Paulo de Góes, UFRJ, Ilha do Fundão, Rio de Janeiro, 21941-590, Brazil

The secreted phosphatase activities of two trypanosomatid parasites were characterized and compared with supernatants of living cells. The plant parasite *Phytomonas francai* and the phytophagous hemipteran parasite *Herpetomonas* sp. hydrolyzed p-nitrophenylphosphate at a rate of 15.54 and 6.51 nmol Pi/mg of protein per min, respectively. Sodium orthovanadate (N(a)VO(3)) and sodium fluoride (NaF) decreased the phosphatase activities. The phosphatase activity of *P. francai* was drastically diminished (73 percent inhibition) in the presence of sodium tartrate, whereas the phosphatase activity of *Herpetomonas* sp. was inhibited by 23 percent. Cytochemical analysis showed the localization of these enzymes on the external surface and in the flagellar pocket of the two trypanosomatids. Sodium tartrate inhibited this reaction, confirming the biochemical data. Platelet-activating factor modulated the phosphatase activities, inhibiting *P. francai* activity and stimulating *Herpetomonas* sp. phosphatase activity.

14923. **Farr, H. & Gull, K., 2009.** Functional studies of an evolutionarily conserved, cytochrome b5 domain protein reveal a specific role in axonemal organisation and the general phenomenon of post-division axonemal growth in trypanosomes. *Cell Motility and the Cytoskeleton*, **66** (1): 24-35.

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Eukaryotic cilia and flagella are highly conserved structures composed of a canonical 9+2 microtubule axoneme. Several recent proteomic studies of cilia and flagella have been published, including a proteome of the flagellum of the protozoan parasite *Trypanosoma brucei*. Comparing proteomes reveals many novel proteins that appear to be widely conserved in evolution. Amongst these, we found a previously uncharacterised protein which localised to the axoneme in *T. brucei*, and therefore named it trypanosome axonemal protein (TAX)-2. Ablation of the protein using RNA interference in the procyclic form of the parasite has no effect on growth but causes a reduction in motility. Using transmission electron microscopy, various structural defects were seen in some axonemes, most frequently with microtubule doublets missing from the 9+2 arrangement. RNAi knockdown of TAX-2 expression in the bloodstream form of the parasite caused defects in growth and cytokinesis, a further example of the effects caused by loss of flagellar function in bloodstream form *T. brucei*. In procyclic cells we used a new set of vectors to ablate protein expression in cells

expressing a GFP:TAX-2 fusion protein, which enabled us to easily quantify protein reduction and visualise axonemes made before and after RNAi induction. This establishes a useful generic technique but also revealed a specific observation that the new flagellum on the daughter trypanosome continues growth after cytokinesis. Our results provide evidence for TAX-2 function within the axoneme, where we suggest that it is involved in processes linking the outer doublet microtubules and the central pair.

14924. **Fisk, J. C., Sayegh, J., Zurita-Lopez, C., Menon, S., Presnyak, V., Clarke, S. G. & Read, L. K., 2009.** A type III protein arginine methyltransferase from the protozoan parasite *Trypanosoma brucei*. *Journal of Biological Chemistry*, **284** (17): 11590-11600.

Department of Microbiology and Immunology, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, New York 14124.

Arginine methylation is a widespread post-translational modification of proteins catalyzed by a family of protein arginine methyltransferases (PRMTs). The ancient protozoan parasite, *Trypanosoma brucei*, possesses five putative PRMTs, a relatively large number for a single-celled eukaryote. Trypanosomatids lack gene regulation at the level of transcription, instead relying on post-transcriptional control mechanisms that act at the levels of RNA turnover, translation, and editing, all processes that likely involve multiple RNA-binding proteins, which are common targets of arginine methylation. Here, we report the characterization of a trypanosome PRMT, TbPRMT7, which is homologous to human PRMT7. Interestingly, trypanosomatids are the only single-celled eukaryotes known to harbour a PRMT7 homologue. TbPRMT7 differs dramatically from all known metazoan PRMT7 homologues in lacking the second AdoMet binding-like domain that is required for activity of the human enzyme. Nevertheless, bacterially expressed TbPRMT7 exhibits robust methyltransferase activity toward multiple targets *in vitro*. High resolution ion exchange chromatography analysis of methylated substrates reveals that TbPRMT7 is a type III PRMT, catalyzing the formation of only monomethylarginine, thereby representing the only exclusively type III PRMT identified to date. TbPRMT7 is expressed in both mammalian and insect stage *T. brucei* and is apparently dispensable for growth in both life cycle stages. The enzyme is cytoplasmically localized and is a component of several higher order complexes *in vivo*. Together, our studies indicate that TbPRMT7 is a Type III PRMT, and its robust activity and presence in numerous complexes suggest it plays multiple roles during the complex *T. brucei* life cycle.

14925. **Forsythe, G. R., McCulloch, R. & Hammarton, T. C., 2009.** Hydroxyurea-induced synchronisation of bloodstream stage *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **164** (2): 131-136.

Division of Infection & Immunity and Wellcome Centre for Molecular Parasitology, Glasgow Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow, Scotland, UK.

Synchronisation of the *Trypanosoma brucei* cell cycle proved elusive for many years. A recent report demonstrated that synchronisation of procyclic form cells was possible following treatment with hydroxyurea. Here, that work is extended to the disease-relevant,

mammalian-infective bloodstream stage trypanosome. Treatment of bloodstream stage Lister 427 *T. brucei* cells growing *in vitro* with 10 µg ml⁻¹ hydroxyurea for 6h led to an enrichment of cells in S phase. Following removal of the drug, cells proceeded uniformly through one round of the cell cycle, providing a much needed tool to enrich for specific cell cycle stages, in a manner similar to hydroxyurea treatment of procyclic form *T. brucei*.

14926. **Gawryluk, R. M. & Gray, M. W., 2009.** A split and rearranged nuclear gene encoding the iron-sulphur subunit of mitochondrial succinate dehydrogenase in *Euglenozoa*. *BMC Research Notes*, 2: 16.

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Analyses based on phylogenetic and ultrastructural data have suggested that euglenids (such as *Euglena gracilis*), trypanosomatids and diplomonads are members of a monophyletic lineage termed Euglenozoa. However, many uncertainties are associated with phylogenetic reconstructions for ancient and rapidly evolving groups; thus, rare genomic characters become increasingly important in reinforcing inferred phylogenetic relationships. We discovered that the iron-sulphur subunit (SdhB) of mitochondrial succinate dehydrogenase is encoded by a split and rearranged nuclear gene in *Euglena gracilis* and trypanosomatids, an example of a rare genomic character. The two subgenic modules are transcribed independently and the resulting mRNAs appear to be independently translated, with the two protein products imported into mitochondria, based on the presence of predicted mitochondrial targeting peptides. Although the inferred protein sequences are in general very divergent from those of other organisms, all of the required iron-sulphur cluster-coordinating residues are present. Moreover, the discontinuity in the euglenozoan SdhB sequence occurs between the two domains of a typical, covalently continuous SdhB, consistent with the inference that the euglenozoan “half” proteins are functional. The discovery of this unique molecular marker provides evidence for the monophyly of *Euglenozoa* that is independent of evolutionary models. Our results pose questions about the origin and timing of this novel gene arrangement and the structure and function of euglenozoan SdhB.

14927. **Greig, N., Wyllie, S., Patterson, S. & Fairlamb, A. H., 2009.** A comparative study of methylglyoxal metabolism in trypanosomatids. *Febs Journal*, 276 (2): 376-386.

Division of Biological Chemistry and Drug Discovery, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, UK.

The glyoxalase system, comprising the metalloenzymes glyoxalase I (GLO1) and glyoxalase II (GLO2), is an almost universal metabolic pathway involved in the detoxification of the glycolytic by-product methylglyoxal to d-lactate. In contrast to the situation with the trypanosomatid parasites *Leishmania major* and *Trypanosoma cruzi*, this trypanothione-dependent pathway is less well understood in the African trypanosome, *Trypanosoma brucei*. Although this organism possesses a functional GLO2, no apparent GLO1 gene could be identified in the *T. brucei* genome. The absence of GLO1 in *T. brucei* was confirmed by the lack of GLO1 activity in whole cell extracts, failure to detect a GLO1-like protein on immunoblots of cell lysates, and lack of d-lactate formation from methylglyoxal as compared to *L. major* and *T. cruzi*. *T. brucei* procyclics were found to be

2.4-fold and 5.7-fold more sensitive to methylglyoxal toxicity than *T. cruzi* and *L. major*, respectively. *T. brucei* also proved to be the least adept of the “Tritryp” parasites in metabolizing methylglyoxal, producing l-lactate rather than d-lactate. Restoration of a functional glyoxalase system by expression of *T. cruzi* GLO1 in *T. brucei* resulted in increased resistance to methylglyoxal and increased conversion of methylglyoxal to d-lactate, demonstrating that GLO2 is functional *in vivo*. Procyclic forms of *T. brucei* possess NADPH-dependent methylglyoxal reductase and NAD(+)-dependent l-lactaldehyde dehydrogenase activities sufficient to account for all of the methylglyoxal metabolized by these cells. We propose that the predominant mechanism for methylglyoxal detoxification in the African trypanosome is via the methylglyoxal reductase pathway to l-lactate.

14928. **Harrington, J. M., Howell, S. & Hajduk, S. L., 2009.** Membrane permeabilization by trypanosome lytic factor, a cytolytic human high-density lipoprotein. *Journal of Biological Chemistry*, **284**(20): 13505-13512.

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Trypanosome lytic factor (TLF) is a subclass of human high-density lipoprotein (HDL) that mediates an innate immune killing of certain mammalian trypanosomes, most notably *Trypanosoma brucei brucei*, the causative agent of a wasting disease in cattle. Mechanistically, killing is initiated in the lysosome of the target trypanosome where the acidic pH facilitates a membrane disrupting activity by TLF. Here we utilize a model liposome system to characterize the membrane binding and permeabilizing activity of TLF and its protein constituents, haptoglobin related protein (Hpr), apolipoprotein L-1 (apoL-1) and apolipoprotein A-1 (apoA-1). We show that TLF efficiently binds and permeabilizes unilamellar liposomes at lysosomal pH whereas non-lytic human HDL exhibits inefficient permeabilizing activity. Purified, delipidated Hpr and apoL-1 both efficiently permeabilize lipid bilayers at low pH. Trypanosome lytic factor, apoL-1 and apoA-1 exhibit specificity for anionic membranes while Hpr permeabilizes both anionic and zwitterionic membranes. Analysis of the relative particle sizes of susceptible liposomes reveals distinctly different membrane-active behaviour for native TLF and the delipidated protein components. We propose that lysosomal membrane damage in TLF susceptible trypanosomes is initiated by the stable association of the TLF particle with the lysosomal membrane and that this is a property unique to this subclass of human HDL.

14929. **Hart, S. R., Lau, K. W., Hao, Z., Broadhead, R., Portman, N., Huhmer, A., Gull, K., McKean, P. G., Hubbard, S. J. & Gaskell, S. J., 2009.** Analysis of the trypanosome flagellar proteome using a combined electron transfer/collisionally activated dissociation strategy. *Journal of the American Society of Mass Spectrometry*, **20** (2): 167-175.

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The use of electron-transfer dissociation as an alternative peptide ion activation method for generation of protein sequence information is examined here in comparison with

the conventional method of choice, collisionally activated dissociation, using a linear ion trapping instrument. Direct comparability between collisionally and electron-transfer-activated product ion data was ensured by employing an activation-switching method during acquisition, sequentially activating precisely the same precursor ion species with each fragmentation method in turn. Sequest (Thermo Fisher Scientific, San Jose, CA) searching of product ion data generated an overlapping yet distinct pool of polypeptide identifications from the products of collisional and electron-transfer-mediated activation. To provide a highly confident set of protein recognitions, identification data were filtered using parameters that achieved a peptide false discovery rate of 1 percent, with two or more independent peptide assignments required for each protein. The use of electron transfer dissociation (ETD) has allowed us to identify additional peptides where the quality of product ion data generated by collisionally activated dissociation (CAD) was insufficient to infer peptide sequence. Thus, a combined ETD/CAD approach leads to the recognition of more peptides and proteins than are achieved using peptide analysis by CAD- or ETD-based tandem mass spectrometry alone.

14930. **Helm, J. R., Wilson, M. E. & Donelson, J. E., 2009.** Differential expression of a protease gene family in African trypanosomes. *Molecular and Biochemical Parasitology*, **163** (1): 8-18.

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During their life cycle African trypanosomes must quickly adapt to the different environments of the tsetse fly midgut and the mammalian bloodstream by modulating expression of many of their genes. One group of these differentially expressed genes encodes different forms of a major surface protease. Using a luciferase reporter gene transiently or permanently transfected into trypanosomes, we show here that the 3'-UTRs of these protease genes are responsible for their differential expression. Deletion analysis of the 389-bp 3'-UTR of one of the protease genes, MSP-B, demonstrated that it contains a U-rich regulatory region of about 23bp (UCGUCUGUUAUUUCUUAGUCCAG), which suppresses expression of the reporter protein in bloodstream trypanosomes by as much as 25-fold, but has little effect on the reporter expression in procyclic (tsetse fly) trypanosomes. Replacing the entire 3'-UTR with just this 23-bp element mimicked most of the suppression effect of the complete 3'-UTR. Northern blots showed that the 23-bp element influences the steady state RNA level, but not enough to account for the 25-fold suppression effect. Polysome analyses showed that in procyclic trypanosomes more of the total protease mRNA is associated with intermediate-sized and large polysomes than in bloodstream trypanosomes. Thus, the 23-bp element of this protease gene affects both the level of RNA and its translation.

14931. **Hertz-Fowler, C., Figueiredo, L. M., Quail, M. A., Becker, M., Jackson, A., Bason, N., Brooks, K., Churcher, C., Fahkro, S., Goodhead, I., Heath, P., Kartvelishvili, M., Mungall, K., Harris, D., Hauser, H., Sanders, M., Saunders, D., Seeger, K., Sharp, S., Taylor, J. E., Walker, D., White, B., Young, R., Cross, G. A., Rudenko, G., Barry, J. D., Louis, E. J. & Berriman, M., 2008.** Telomeric expression sites are highly conserved in *Trypanosoma brucei*. *PLoS ONE*, **3** (10): e3527.

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Sub-telomeric regions are often under-represented in genome sequences of eukaryotes. One of the best known examples of the use of telomere proximity for adaptive purposes are the bloodstream expression sites (BESs) of the African trypanosome *Trypanosoma brucei*. To enhance our understanding of BES structure and function in host adaptation and immune evasion, the BES repertoire from the Lister 427 strain of *T. brucei* was independently tagged and sequenced. BESs are polymorphic in size and structure but reveal a surprisingly conserved architecture in the context of extensive recombination. Very small BESs do exist and many functioning BESs do not contain the full complement of expression site associated genes (ESAGs). The consequences of duplicated or missing ESAGs, including ESAG9, a newly named ESAG12, and additional variant surface glycoprotein genes (VSGs) were evaluated by functional assays after BESs were tagged with a drug-resistance gene. Phylogenetic analysis of constituent ESAG families suggests that BESs are sequence mosaics and that extensive recombination has shaped the evolution of the BES repertoire. This work opens important perspectives in understanding the molecular mechanisms of antigenic variation, a widely used strategy for immune evasion in pathogens, and telomere biology.

14932. **Holzmuller, P., Grebaut, P., Peltier, J. B., Brizard, J. P., Perrone, T., Gonzatti, M., Bengaly, Z., Rossignol, M., Aso, P. M., Vincendeau, P., Cuny, G., Boulange, A. & Frutos, R., 2008.** Secretome of animal trypanosomes. *Annals of the New York Academy of Sciences*, **1149**: 337-342.

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Animal trypanosomosis is one of the most severe constraints to agricultural development in sub-Saharan Africa and is also an important disease of livestock in Latin America and Asia. The causative agents are various species of protozoan parasites belonging to the genus *Trypanosoma*, among which *T. congolense* and *T. evansi* are the major pathogenic species. The extracellular position of trypanosomes obliges us to consider both the parasite and its excreted/secreted factors in the course of the physiopathologic process. The advent of proteomics led us to propose a comparative approach of the proteome (i.e. the whole parasite content) and the secretome (i.e., naturally excreted/secreted molecules) of *T. congolense* and *T. evansi* with particular attention to common and specific molecules between strains of differing virulence and pathogenicity. The molecular identification of differentially expressed trypanosome molecules correlated with either the virulence process or the pathogenicity will provide new potential molecular targets for improved field diagnosis and chemotherapy of animal trypanosomosis.

14933. **Hury, A., Goldshmidt, H., Tkacz, I. D. & Michaeli, S., 2009.** Trypanosome spliced-leader-associated RNA (SLA1) localization and implications for spliced-leader RNA biogenesis. *Eukaryotic Cell*, **8** (1): 56-68.

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Spliced-leader-associated RNA (SLA1) guides the pseudouridylation at position -12 (relative to the 5' splice site) of the spliced-leader (SL) RNA in all trypanosomatid species. Nevertheless, the exact role of this RNA is currently unknown. Here, we demonstrate that the absence of pseudouridine on *Leptomonas collosoma* SL RNA has only a minor effect on the ability of this RNA to function in trans splicing *in vivo*. To investigate the possible role of SLA1 during SL RNA biogenesis, the structure of the SL RNA was examined in permeable *Trypanosoma brucei* cells depleted for CBF5, the H/ACA pseudouridine synthase, lacking SLA1. Our results suggest that in the absence of SLA1, the SL RNA secondary structure is changed, as was detected by differential sensitivity to oligonucleotide-directed RNase H cleavage, suggesting that the association of SLA1 maintains the SL RNA in a structural form which is distinct from the structure of the SL RNA in the steady state. In *T. brucei* cells depleted for the SL RNA core protein SmD1, SL RNA first accumulates in large amounts in the nucleus and then is expelled to the cytoplasm. Here, we demonstrate by *in vivo* aminomethyltrimethyl UV cross-linking studies that under SmD1 depletion, SLA1 remains bound to SL RNA and escorts the SL RNA to the cytoplasm. *In situ* hybridization with SLA1 and SL RNA demonstrates colocalization between SLA1 and the SL RNA transcription factor tSNAP42, as well as with Sm proteins, suggesting that SLA1 associates with SL RNA early in its biogenesis. These results demonstrate that SLA1 is a unique chaperonic RNA that functions during the early biogenesis of SL RNA to maintain a structure that is most probably suitable for cap 4 modification.

14934. **Izquierdo, L., Atrih, A., Rodrigues, J. A., Jones, D. C. & Ferguson, M. A., 2009.** *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase has unusual substrate specificity and protects the parasite from stress. *Eukaryotic Cell*, **8** (2): 230-240.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

In this paper, we describe the range of N-linked glycan structures produced by wild-type and glucosidase II null mutant bloodstream form *Trypanosoma brucei* parasites and the creation and characterization of a bloodstream form *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase null mutant. These analyses highlight peculiarities of the *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase, including an unusually wide substrate specificity, ranging from Man(5)GlcNAc(2) to Man(9)GlcNAc(2) glycans, and an unusually high efficiency *in vivo*, quantitatively glucosylating the Asn263 N-glycan of variant surface glycoprotein (VSG) 221 and 75 percent of all non-VSG N glycosylation sites. We also show that although *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase is not essential for parasite growth at 37 °C, it is essential for parasite growth and survival at 40 °C. The null mutant was also shown to be hypersensitive to the effects of the N glycosylation inhibitor tunicamycin. Further analysis of bloodstream form *Trypanosoma brucei* under normal conditions and stress conditions suggests that it does not have a classical unfolded protein response triggered by sensing unfolded proteins in the endoplasmic reticulum. Rather, judging by its uniform Grp78/BiP levels, it appears to have an unregulated and constitutively active endoplasmic reticulum protein folding system. We suggest that the latter may be particularly appropriate for this organism, which has an extremely high flux of glycoproteins through its secretory pathway.

14935. **Jackson, A. P., Quail, M. A. & Berriman, M., 2008.** Insights into the genome sequence of a free-living Kinetoplastid: *Bodo saltans* (Kinetoplastida: Euglenozoa). *BMC Genomics*, **9**: 594.

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Bodo saltans is a free-living kinetoplastid and among the closest relatives of the trypanosomatid parasites, which cause such human diseases as African sleeping sickness, leishmaniasis and Chagas disease. A *B. saltans* genome sequence will provide a free-living comparison with parasitic genomes necessary for comparative analyses of existing and future trypanosomatid genomic resources. Various coding regions were sequenced to provide a preliminary insight into the bodonid genome sequence relative to trypanosomatid sequences. 0.4 Mbp of *B. saltans* genome were sequenced from 12 distinct regions and contained 178 coding sequences. As in trypanosomatids, introns were absent and percentGC was elevated in coding regions, greatly assisting in gene finding. In the regions studied, roughly 60 percent of all genes had homologues in trypanosomatids, while 28 percent were *Bodo*-specific. Intergenic sequences were typically short, resulting in higher gene density than in trypanosomatids. Although synteny was typically conserved for those genes with trypanosomatid homologues, strict co-linearity was rarely observed because gene order was regularly disrupted by *Bodo*-specific genes. The results show that the *B. saltans* genome contains both sequences homologous to trypanosomatids and sequences never seen before. Structural similarities suggest that its assembly should be solvable, and, although *de novo* assembly will be necessary, existing trypanosomatid projects will provide some guide to annotation. A complete genome sequence will provide an effective ancestral model for understanding the shared and derived features of known trypanosomatid genomes, but it will also identify those kinetoplastid genome features lost during the evolution of parasitism.

14936. **Jetton, N., Rothberg, K. G., Hubbard, J. G., Wise, J., Li, Y., Ball, H. L. & Ruben, L., 2009.** The cell cycle as a therapeutic target against *Trypanosoma brucei*: hesperadin inhibits Aurora kinase-1 and blocks mitotic progression in bloodstream forms. *Molecular Microbiology*, **72** (2): 442-458.

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Aurora kinase family members co-ordinate a range of events associated with mitosis and cytokinesis. Anti-cancer therapies are currently being developed against them. Here, we evaluate whether Aurora kinase-1 (*TbAUK1*) from pathogenic *Trypanosoma brucei* might be targeted in anti-parasitic therapies as well. Conditional knockdown of *TbAUK1* within infected mice demonstrated its essential contribution to infection. An *in vitro* kinase assay was developed which used recombinant trypanosome histone H3 as a substrate. Tandem mass spectroscopy identified a novel phosphorylation site in the carboxyl-tail of recombinant trypanosome histone H3. Hesperadin, an inhibitor of human Aurora B, prevented the phosphorylation of substrate with IC(50) of 40 nM. Growth of cultured bloodstream forms was also sensitive to Hesperadin (IC(50) of 50 nM). Hesperadin blocked nuclear division and cytokinesis but not other aspects of the cell cycle. Consequently, growth arrested cells accumulated multiple kinetoplasts, flagella and nucleoli, similar to the effects of RNAi-

dependent knockdown of *TbAUK1* in cultured bloodstream forms cells. Molecular models predicted high-affinity binding of Hesperadin to both conserved and novel sites in *TbAUK1*. Collectively, these data demonstrate that cell cycle progression is essential for infections with *T. brucei* and that parasite Aurora kinases can be targeted with small-molecule inhibitors.

14937. **Kawahara, T., Siegel, T. N., Ingram, A. K., Alsford, S., Cross, G. A. & Horn, D., 2008.** Two essential MYST-family proteins display distinct roles in histone H4K10 acetylation and telomeric silencing in trypanosomes. *Molecular Microbiology*, **69** (4): 1054-1068.

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Chromatin modification is important for virtually all aspects of DNA metabolism but little is known about the consequences of such modification in trypanosomatids, early branching protozoa of significant medical and veterinary importance. MYST-family histone acetyltransferases in other species function in transcription regulation, DNA replication, recombination and repair. *Trypanosoma brucei* HAT3 was recently shown to acetylate histone H4K4 and we now report characterization of all three *T. brucei* MYST acetyltransferases (HAT1-3). First, GFP-tagged HAT1-3 all localize to the trypanosome nucleus. While HAT3 is dispensable, both HAT1 and HAT2 are essential for growth. Strains with HAT1 knock-down display mitosis without nuclear DNA replication and also specific de-repression of a telomeric reporter gene, a rare example of transcription control in an organism with widespread and constitutive polycistronic transcription. Finally, we show that HAT2 is responsible for H4K10 acetylation. By analogy to the situation in *Saccharomyces cerevisiae*, we discuss low-level redundancy of acetyltransferase function in *T. brucei* and suggest that two MYST-family acetyltransferases are essential due to the absence of a Gen5 homologue. The results are also consistent with the idea that HAT1 contributes to establishing boundaries between transcriptionally active and repressed telomeric domains in *T. brucei*.

14938. **Lee, J. H., Jung, H. S. & Gunzl, A., 2009.** Transcriptionally active TFIID of the early-diverged eukaryote *Trypanosoma brucei* harbours two novel core subunits but not a cyclin-activating kinase complex. *Nucleic Acids Research*. **Advanced Access, published on line, April 22.**

Department of Genetics and Developmental Biology, Department of Molecular, Microbial and Structural Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3301 and Department of Cell Biology, University of Massachusetts, 55 Lake Ave, North, Worcester, MA 01655, USA.

Trypanosoma brucei is a member of the early-diverged, protistan family *Trypanosomatidae* and a lethal parasite causing African sleeping sickness in humans. Recent studies revealed that *T. brucei* harbours extremely divergent orthologues of the general transcription factors TBP, TFIIA, TFIIB and TFIID and showed that these factors are essential for initiating RNA polymerase II-mediated synthesis of spliced leader (SL) RNA, a trans splicing substrate and key molecule in trypanosome mRNA maturation. In yeast and metazoans, TFIID is composed of a core of seven conserved subunits and the ternary cyclin-

activating kinase (CAK) complex. Conversely, only four TFIIF subunits have been identified in *T. brucei*. Here, we characterize the first protistan TFIIF which was purified in its transcriptionally active form from *T. brucei* extracts. The complex consisted of all seven core subunits but lacked the CAK sub-complex; instead it contained two trypanosomatid-specific subunits, which were indispensable for parasite viability and SL RNA gene transcription. These findings were corroborated by comparing the molecular structures of trypanosome and human TFIIF. While the ring-shaped core domain was surprisingly congruent between the two structures, trypanosome TFIIF lacked the knob-like CAK moiety and exhibited extra densities on either side of the ring, presumably due to the specific subunits.

14939. **Leite, M. S., Thomaz, R., Oliveira, J. H., Oliveira, P. L. & Meyer-Fernandes, J. R., 2009.** *Trypanosoma brucei brucei*: effects of ferrous iron and haeme on ecto-nucleoside triphosphate diphosphohydrolase activity. *Experimental Parasitology*, **121** (2): 137-143.

Laboratório de Bioquímica Celular, Instituto de Bioquímica Medica, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, UFRJ, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, Brazil.

Trypanosoma brucei brucei is the causative agent of animal African trypanosomiasis, also called nagana. Procyclic vector form resides in the midgut of the tsetse fly, which feeds exclusively on blood. Haemoglobin digestion occurs in the midgut resulting in an intense release of free haeme. In the present study we show that the magnesium-dependent ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) activity of procyclic *T. brucei brucei* is inhibited by ferrous iron and haeme. The inhibition of E-NTPDase activity by ferrous iron, but not by haeme, was prevented by pre-incubation of cells with catalase. However, antioxidants that permeate cells, such as PEG-catalase and N-acetyl-cysteine prevented the inhibition of E-NTPDase by haeme. Ferrous iron was able to induce an increase in lipid peroxidation, while haeme did not. Therefore, both ferrous iron and haeme can inhibit E-NTPDase activity of *T. brucei brucei* by means of formation of reactive oxygen species, but apparently acting through distinct mechanisms.

14940. **Maia da Silva, F., Marcili, A., Lima, L., Cavazzana, M., Jr., Ortiz, P. A., Campaner, M., Takeda, G. F., Paiva, F., Nunes, V. L., Camargo, E. P. & Teixeira, M. M., 2009.** *Trypanosoma rangeli* isolates of bats from Central Brazil: genotyping and phylogenetic analysis enable description of a new lineage using spliced-leader gene sequences. *Acta Tropica*, **109** (3): 199-207.

Departamento de Parasitologia, Universidade de São Paulo, São Paulo, SP, Brazil.

Trypanosoma rangeli infects several mammalian orders but has never confidently been described in *Chiroptera*, which are commonly parasitized by many trypanosome species. Here, we described trypanosomes from bats captured in Central Brazil identified as *T. rangeli*, *T. dionisii*, *T. cruzimarinkellei* and *T. cruzi*. Two isolates, Tra643 from *Platyrrhinus lineatus* and Tra1719 from *Artibeus planirostris* were identified as *T. rangeli* by morphological, biological and molecular methods, and confirmed by phylogenetic analyses. Analysis using SSU rDNA sequences clustered these bat trypanosomes together with *T.*

rangeli from other hosts, and separated them from other trypanosomes from bats. Genotyping based on length and sequence polymorphism of PCR-amplified intergenic spliced-leader gene sequences assigned Tra1719 to the lineage A whereas Tra643 was shown to be a new genotype and was assigned to the new lineage E. To our knowledge, these two isolates are the earliest *T. rangeli* from bats and the first isolates from Central Brazil molecularly characterized. *Rhodnius stali* captured for this study was found infected by *T. rangeli* and *T. cruzi*.

14941. **Marin, C., Dollet, M., Pages, M. & Bastien, P., 2009.** Large differences in the genome organization of different plant Trypanosomatid parasites (*Phytomonas* sp.) reveal wide evolutionary divergences between taxa. *Infection, Genetics and Evolution*, **9** (2): 235-240.

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All currently known plant trypanosomes have been grouped in the genus *Phytomonas* sp., although they can differ greatly in terms of both their biological properties and effects upon the host. Those parasitizing the phloem sap are specifically associated with lethal syndromes in Latin America, such as, phloem necrosis of coffee, "Heart rot" of coconut and "Marchitez sorpresiva" of oil palm, that inflict considerable economic losses in endemic countries. The genomic organization of one group of *Phytomonas* (D) considered as representative of the genus has been published previously. The present work presents the genomic structure of two representative isolates from the pathogenic phloem-restricted group (H) of *Phytomonas*, analyzed by pulsed field gel electrophoresis followed by hybridization with chromosome-specific DNA markers. It came as a surprise to observe an extremely different genomic organization in this group as compared with that of group D. Most notably, the chromosome number is 7 in this group (with a genome size of 10 Mb) versus 21 in the group D (totalling 25 Mb). These data unravel an unsuspected genomic diversity within plant trypanosomatids that may justify a further debate about their division into different genera.

14942. **Monnerat, S., Clucas, C., Brown, E., Mottram, J. C. & Hammarton, T. C., 2009.** Searching for novel cell cycle regulators in *Trypanosoma brucei* with an RNA interference screen. *BMC Research Notes*, **2**: 46.

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The protozoan parasite *Trypanosoma brucei* is spread by the tsetse fly and causes human African trypanosomiasis. Its cell cycle is complex and not fully understood at the molecular level. The *T. brucei* genome contains over 6 000 protein coding genes with >50 percent having no predicted function. A small scale RNA interference (RNAi) screen was carried out in *Trypanosoma brucei* to evaluate the prospects for identifying novel cycle regulators. Procyclic form *T. brucei* was transfected with a genomic RNAi library and 204 clones isolated. However, only 76 RNAi clones were found to target a protein coding gene of potential interest. These clones were screened for defects in proliferation and cell cycle progression following RNAi induction. Sixteen clones exhibited proliferation defects upon

RNAi induction, with eight clones displaying potential cell cycle defects. To confirm the phenotypes, new RNAi cell lines were generated and characterised for five genes targeted in these clones. While we confirmed that the targeted genes are essential for proliferation, we were unable to unambiguously classify them as cell cycle regulators. Our study identified genes essential for proliferation, but did not, as hoped, identify novel cell cycle regulators. Screening of the RNAi library for essential genes was extremely labour-intensive, which was compounded by the suboptimal quality of the library. For such a screening method to be viable for a large scale or genome wide screen, a new, significantly improved RNAi library will be required, and automated phenotyping approaches will need to be incorporated.

14943. **Mugasa, C. M., Laurent, T., Schoone, G. J., Kager, P. A., Lubega, G. W. & Schallig, H. D., 2009.** Nucleic acid sequence-based amplification with oligochromatography for detection of *Trypanosoma brucei* in clinical samples. *Journal of Clinical Microbiology*, **47** (3): 630-635.

Department of Veterinary Parasitology and Microbiology, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda.

Molecular tools such as real-time nucleic acid sequence-based amplification (NASBA) and PCR have been developed to detect *Trypanosoma brucei* parasites in blood for the diagnosis of human African trypanosomiasis (HAT). Despite good sensitivity, these techniques are not implemented in HAT control programs due to the high cost of the equipment, which is unaffordable for laboratories in developing countries where HAT is endemic. In this study, a simplified technique, oligochromatography (OC), was developed for the detection of amplification products of *T. brucei* 18S rRNA by NASBA. The *T. brucei* NASBA-OC test has analytical sensitivities of 1- 10 parasites/ml on nucleic acids extracted from parasite culture and 10 parasites/ml on spiked blood. The test showed no reaction with nontarget pathogens or with blood from healthy controls. Compared with the composite standard applied in the present study, i.e., parasitological confirmation of a HAT case by direct microscopy or by microscopy after concentration of parasites using either a microhaematocrit centrifugation technique or a mini-anion-exchange centrifugation technique, NASBA-OC on blood samples had a sensitivity of 73.0 percent (95 percent confidence interval, 60 to 83 percent), while standard expert microscopy had a sensitivity of 57.1 percent (95 percent confidence interval, 44 to 69 percent). On cerebrospinal fluid samples, NASBA-OC had a sensitivity of 88.2 percent (95 percent confidence interval, 75 to 95 percent) and standard microscopy had a sensitivity of 86.2 percent (95 percent confidence interval, 64 to 88 percent). The *T. brucei* NASBA-OC test developed in this study can be employed in field laboratories, because it does not require a thermocycler; a simple heat block or a water bath maintained at two different temperatures is sufficient for amplification.

14944. **Myslyuk, I., Doniger, T., Horesh, Y., Hury, A., Hoffer, R., Ziporen, Y., Michaeli, S. & Unger, R., 2008.** Psiscan: a computational approach to identify H/ACA-like and AGA-like non-coding RNA in trypanosomatid genomes. *BMC Bioinformatics*, **9**: 471.

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Detection of non coding RNA (ncRNA) molecules is a major bioinformatics challenge. This challenge is particularly difficult when attempting to detect H/ACA molecules which are involved in converting uridine to pseudouridine on rRNA in trypanosomes, because these organisms have unique H/ACA molecules (termed H/ACA-like) that lack several of the features that characterize H/ACA molecules in most other organisms. We present here a computational tool called Psiscan, which was designed to detect H/ACA-like molecules in trypanosomes. We started by analyzing known H/ACA-like molecules and characterized their crucial elements both computationally and experimentally. Next, we set up constraints based on this analysis and additional phylogenetic and functional data to rapidly scan three trypanosome genomes (*T. brucei*, *T. cruzi* and *L. major*) for sequences that observe these constraints and are conserved among the species. In the next step, we used minimal energy calculation to select the molecules that are predicted to fold into a lowest energy structure that is consistent with the constraints. In the final computational step, we used a Support Vector Machine that was trained on known H/ACA-like molecules as positive examples and on negative examples of molecules that were identified by the computational analyses but were shown experimentally not to be H/ACA-like molecules. The leading candidate molecules predicted by the SVM model were then subjected to experimental validation. The experimental validation showed 11 molecules to be expressed (4 out of 25 in the intermediate stage and 7 out of 19 in the final validation after the machine learning stage). Five of these 11 molecules were further shown to be *bona fide* H/ACA-like molecules. As snoRNA in trypanosomes are organized in clusters, the new H/ACA-like molecules could be used as starting points to manually search for additional molecules in their neighbourhood. All together this study increased our repertoire by fourteen H/ACA-like and six C/D snoRNAs molecules from *T. brucei* and *L. major*. In addition the experimental analysis revealed that six ncRNA molecules that are expressed are not downregulated in CBF5 silenced cells, suggesting that they have structural features of H/ACA-like molecules but do not have their standard function. We termed this novel class of molecules AGA-like, and we are exploring their function. This study demonstrates the power of tight collaboration between computational and experimental approaches in a combined effort to reveal the repertoire of ncRNA molecules.

14945. **Nett, I. R., Martin, D. M., Miranda-Saavedra, D., Lamont, D., Barber, J. D., Mehlert, A. & Ferguson, M. A., 2009.** The phosphoproteome of bloodstream form *Trypanosoma brucei*, causative agent of African sleeping sickness. *Molecular and Cellular Proteomics*. **e Publication ahead of print, April 4.**

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The protozoan parasite *Trypanosoma brucei* is the causative agent of human African sleeping sickness and related animal diseases and it has over 170 predicted protein kinases. Protein phosphorylation is a key regulatory mechanism for cellular function that, thus far, has been studied in *T. brucei* principally through putative kinase mRNA knockdown and observation of the resulting phenotype. However, despite the relatively large kinome of this organism, and the demonstrated essentiality of several *T. brucei* kinases, only 8 specific phosphorylation sites have been determined in this organism. Using a gel-free, phosphopeptide-enrichment, based proteomics approach we have performed the first large-scale phosphorylation site analyses for *T. brucei*. Serine, threonine and tyrosine

phosphorylation sites were determined for a cytosolic protein fraction of the bloodstream form of the parasite, resulting in the identification of 491 phosphoproteins based on the identification of 852 unique phosphopeptides and 1 204 phosphorylation sites. The phosphoproteins detected in this study are predicted from their genome annotations to participate in a wide variety of biological processes, including signal transduction, processing of DNA and RNA, protein synthesis and degradation and to a minor extent in metabolic pathways. The analysis of phosphopeptides and phosphorylation sites was facilitated by in-house developed software and this automated approach was validated by manual annotation of spectra of the kinase subset of proteins. Analysis of the cytosolic bloodstream form *T. brucei* kinome revealed the presence of 44 phosphorylated protein kinases in our dataset, which could be classified into the major eukaryotic protein kinase groups by applying a multi-level hidden Markov model (HMM) library of the kinase catalytic domain. Identification of the kinase phosphorylation sites showed conserved phosphorylation sequence motifs in several kinase activation segments, which supports the view that phosphorylation-based signalling is a general and fundamental regulatory process that extends to this highly divergent lower eukaryote.

14946. **Niemann, M., Kaibel, H., Schluter, E., Weitzel, K., Brecht, M. & Goring, H. U., 2009.** Kinetoplastid RNA editing involves a 3' nucleotidyl phosphatase activity. *Nucleic Acids Research*, **37** (6): 1897-1906.

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Mitochondrial pre-messenger RNAs (pre-mRNAs) in African trypanosomes require RNA editing in order to mature into functional transcripts. The process involves the addition and/or removal of U nucleotides and is mediated by a high-molecular-mass complex, the editosome. Editosomes catalyze the reaction through an enzyme-driven pathway that includes endo/exoribonuclease, terminal uridylyl transferase and RNA ligase activities. Here we show that editing involves an additional reaction step, a 3' nucleotidyl phosphatase activity. The activity is associated with the editing complex and we demonstrate that the editosomal proteins *TbMP99* and *TbMP100* contribute to the activity. Both polypeptides contain endo-exonuclease-phosphatase domains and we show that gene ablation of either one of the two polypeptides is compensated by the other protein. However, simultaneous knockdown of both genes results in trypanosome cells with reduced 3' nucleotidyl phosphatase and reduced editing activity. The data provide a rationale for the *exoUase* activity of the editosomal protein *TbMP42*, which generates nonligatable 3' phosphate termini. Opposing phosphates at the two pre-mRNA cleavage fragments likely function as a roadblock to prevent premature ligation.

14947. **Pereira, F. M., Bernardo, P. S., Dias Junior, P. F., Silva, B. A., Romanos, M. T., d'Avila-Levy, C. M., Branquinho, M. H. & Santos, A. L., 2009.** Differential influence of gp63-like molecules in three distinct *Leptomonas* species on the adhesion to insect cells. *Parasitology Research*, **104** (2): 347-353.

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Parasites belonging to the *Leptomonas* genus have been used as model organisms for studying biochemical, cellular, and genetic processes unique to members of the Trypanosomatidae family. In the present study, the cell-associated and extracellular peptidases of three *Leptomonas* species, *Leptomonas collosoma*, *Leptomonas samueli*, and *Leptomonas wallacei*, were assayed and characterized by gelatin-sodium dodecyl sulfate polyacrylamide gel electrophoresis. All parasites released metallopeptidases, whereas no cell-associated proteolytic activity could be detected in the cellular extracts from *L. collosoma*. Western blotting probed with a polyclonal antibody raised against gp63 from *Leishmania amazonensis* revealed two major reactive polypeptides of apparent molecular masses of 63 and 52 kDa, with different intensities in cellular extracts and released proteins from the studied trypanosomatids. Flow cytometry and fluorescence microscopy analyses showed that the gp63-like molecules have a surface location. This is the first report on the presence of gp63-like molecules in *L. collosoma*, *L. samueli*, and *L. wallacei*. The pretreatment of *L. samueli* and *L. wallacei* with anti-gp63 antibody significantly diminished their association index to *Aedes albopictus* cell line (C6/36), suggesting a potential involvement of the gp63-like molecules in the interaction process of these insect trypanosomatids with the vector.

14948. **Prohaska, K. & Williams, N., 2009.** Assembly of the *Trypanosoma brucei* 60S ribosomal subunit nuclear export complex requires trypanosome-specific proteins P34 and P37. *Eukaryotic Cell*, **8** (1): 77-87.

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We previously identified two *Trypanosoma brucei* RNA binding proteins, P34 and P37, and determined that they are essential for proper ribosomal assembly in this organism. Loss of these proteins via RNA interference is lethal and causes a decrease in both 5S rRNA levels and formation of 80S ribosomes, concomitant with a decrease in total cellular protein synthesis. These data suggest that these proteins are involved at some point in the ribosomal biogenesis pathway. In the current study, we have performed subcellular fractionation in conjunction with immune capture experiments specific for 60S ribosomal proteins and accessory factors in order to determine when and where P34 and P37 are involved in the ribosomal biogenesis pathway. These studies demonstrate that P34 and P37 associate with the 60S ribosomal subunit at the stage of the nucleolar 90S particle and remain associated subsequent to nuclear export. In addition, P34 and P37 associate with conserved 60S ribosomal subunit nuclear export factors exportin 1 and Nmd3, suggesting that they are components of the 60S ribosomal subunit nuclear export complex in *T. brucei*. Most significantly, the pre-60S complex does not associate with exportin 1 or Nmd3 in the absence of P34 and P37. These results demonstrate that, although *T. brucei* 60S ribosomal subunits utilize a nuclear export complex similar to that described for other organisms, trypanosome-specific factors are essential to the process.

14949. **Rotureau, B., Morales, M. A., Bastin, P. & Spath, G. F., 2009.** The flagellum-MAP kinase connection in Trypanosomatids: a key sensory role in parasite signaling and development? *Cellular Microbiology*. **e Publication ahead of print, February 4.**

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Trypanosomatid parasites are the causative agents of severe human diseases such as sleeping sickness, Chagas disease and leishmaniasis. These microorganisms are transmitted via different insect vectors and hence are confronted to changing environments during their infectious cycle in which they activate specific and complex patterns of differentiation. Several studies in *Trypanosoma brucei* and in different sub-species of *Leishmania* have shed light on the role of mitogen activated protein (MAP) kinases in these processes. Surprisingly, several MAP kinases turned out to be involved in the control of flagellum length in the promastigote stage of *Leishmania*. Recently, a sensory function has been recognized for cilia and flagella in unicellular and multi-cellular eukaryotes. This review aims to stimulate discussions on the possibility that the Trypanosomatid flagellum could act as a sensory organ through the MAP kinase pathway, with the objective to encourage investigation of this new hypothesis through a series of proposed experimental approaches.

14950. **Sakurai, T., Tanaka, M., Kawazu, S. & Inoue, N., 2009.** Establishment of an *in vitro* transgene expression system in epimastigotes of *Trypanosoma congolense*. *Parasitology International*, **58** (1): 110-113.

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Trypanosoma congolense epimastigote forms (EMFs) adhere to the tsetse fly proboscis, proliferate, and differentiate into animal-infective metacyclic forms (MCFs). This differentiation step, called metacyclogenesis, is indispensable for the cyclical transmission of the parasite. Although an *in vitro* metacyclogenesis culture system was established several decades ago, few genetic tools have been utilized to investigate the molecular mechanisms underlying *T. congolense* metacyclogenesis. This study established a transgene expression system using an *in vitro* derived EMF of *T. congolense* IL3000, and the transgenic EMF successfully underwent metacyclogenesis *in vitro*. The newly constructed expression vector pSAK was designed for integration into the alpha-beta tubulin locus, which is tandemly arranged in the *T. congolense* genome. The expression cassette of pSAK/enhanced green fluorescent protein (eGFP) was transfected into the EMF by electroporation. An EMF expressing eGFP was successfully generated and differentiated into an MCF that constitutively expressed eGFP. The *in vitro* metacyclogenesis system in combination with the transgenic EMF technique will be important tools to investigate the molecular mechanisms of metacyclogenesis.

14951. **Sela, D., Yaffe, N. & Shlomai, J., 2008.** Enzymatic mechanism controls redox-mediated protein-DNA interactions at the replication origin of kinetoplast DNA minicircles. *Journal of Biological Chemistry*, **283** (46): 32034-32044.

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Kinetoplast DNA (kDNA) is the mitochondrial DNA of trypanosomatids. Its major components are several thousand topologically interlocked DNA minicircles. Their replication origins are recognized by universal minicircle sequence-binding protein (UMSBP), a CCHC-type zinc finger protein, which has been implicated with minicircle

replication initiation and kDNA segregation. Interactions of UMSBP with origin sequences *in vitro* have been found to be affected by the protein's redox state. Reduction of UMSBP activates its binding to the origin, whereas UMSBP oxidation impairs this activity. The role of redox in the regulation of UMSBP *in vivo* was studied here in synchronized cell cultures, monitoring both UMSBP origin binding activity and its redox state, throughout the trypanosomatid cell cycle. These studies indicated that UMSBP activity is regulated *in vivo* through the cell cycle dependent control of the protein's redox state. The hypothesis that UMSBP's redox state is controlled by an enzymatic mechanism, which mediates its direct reduction and oxidation, was challenged in a multienzyme reaction, reconstituted with pure enzymes of the trypanosomal major redox-regulating pathway. Coupling *in vitro* of this reaction with a UMSBP origin-binding reaction revealed the regulation of UMSBP activity through the opposing effects of tryparedoxin and tryparedoxin peroxidase. In the course of this reaction, tryparedoxin peroxidase directly oxidizes UMSBP, revealing a novel regulatory mechanism for the activation of an origin-binding protein, based on enzyme-mediated reversible modulation of the protein's redox state. This mode of regulation may represent a regulatory mechanism, functioning as an enzyme-mediated, redox-based biological switch.

14952. **Smith, E. E. & Malik, H. S., 2009.** The apolipoprotein L family of programmed cell death and immunity genes rapidly evolved in primates at discrete sites of host-pathogen interactions. *Genome Research*, **19**(5) 850-858.

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Apolipoprotein L1 (APOL1) is a human protein that confers immunity to *Trypanosoma brucei* infections but can be countered by a trypanosome-encoded antagonist SRA. APOL1 belongs to a family of programmed cell death genes whose proteins can initiate host apoptosis or autophagic death. We report here that all six members of the APOL gene family (APOL1-6) present in humans have rapidly evolved in simian primates. APOL6, furthermore, shows evidence of an adaptive sweep during recent human evolution. In each APOL gene tested, we found rapidly evolving codons in or adjacent to the SRA-interacting protein domain (SID), which is the domain of APOL1 that interacts with SRA. In APOL6, we also found a rapidly changing 13-amino-acid cluster in the membrane-addressing domain (MAD), which putatively functions as a pH sensor and regulator of cell death. We predict that APOL genes are antagonized by pathogens by at least two distinct mechanisms: SID antagonists which include SRA that interact with the SID of various APOL proteins, and MAD antagonists that interact with the MAD hinge base of APOL6. These antagonists either block or prematurely cause APOL-mediated programmed cell death of host cells to benefit the infecting pathogen. These putative interactions must occur inside host cells, in contrast to secreted APOL1 that trafficks to the trypanosome lysosome. Hence, the dynamic APOL gene family appears to be an important link between programmed cell death of host cells and immunity to pathogens.

14953. **Stern, M. Z., Gupta, S. K., Salmon-Divon, M., Haham, T., Barda, O., Levi, S., Wachtel, C., Nilsen, T. W. & Michaeli, S., 2009.** Multiple roles for polypyrimidine tract binding (PTB) proteins in trypanosome RNA metabolism. *Rna*, **15** (4): 648-665.

Bar-Ilan University, Ramat-Gan, Israel.

Trypanosomatid genomes encode for numerous proteins containing an RNA recognition motif (RRM), but the function of most of these proteins in mRNA metabolism is currently unknown. Here, we report the function of two such proteins that we have named PTB1 and PTB2, which resemble the mammalian polypyrimidine tract binding proteins (PTB). RNAi silencing of these factors indicates that both are essential for life. PTB1 and PTB2 reside mostly in the nucleus, but are found in the cytoplasm, as well. Microarray analysis performed on PTB1 and PTB2 RNAi silenced cells indicates that each of these factors differentially affects the transcriptome, thus regulating a different subset of mRNAs. PTB1 and PTB2 substrates were categorized bioinformatically, based on the presence of PTB binding sites in their 5' and 3' flanking sequences. Both proteins were shown to regulate mRNA stability. Interestingly, PTB proteins are essential for trans-splicing of genes containing C-rich polypyrimidine tracts. PTB1, but not PTB2, also affects cis-splicing. The specificity of binding of PTB1 was established *in vivo* and *in vitro* using a model substrate. This study demonstrates for the first time that trans-splicing of only certain substrates requires specific factors such as PTB proteins for their splicing. The trypanosome PTB proteins, like their mammalian homologs, represent multivalent RNA binding proteins that regulate mRNAs from their synthesis to degradation.

14954. **Stevens, J. R., 2008.** Kinetoplastid phylogenetics, with special reference to the evolution of parasitic trypanosomes. *Parasite*, **15** (3): 226-232.

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To fully understand the evolutionary history of parasitic kinetoplastids and to understand the context within which the evolution of each parasite group has developed, an understanding not just of the parasites, but of all kinetoplastids is required. Accordingly, this paper provides an overview of kinetoplastid evolution and systematics, including coverage of the proposal by Moreira *et al.* (2004) to divide kinetoplasts into Prokinetoplastina (Ichthyobodo and Perkinsiella) and Metakinetoplastina (other bodonids and trypanosomatids). The implications of such a revision, with regard to correctly identifying outgroup taxa for studies of evolution within taxa of medical importance, are addressed, together with a more detailed review of the evolution and origins of the trypanosomes in the light of new phylogenies, new approaches and revisions in kinetoplastid systematics.

14955. **Vazquez, M. P., Muallem, D., Bercovich, N., Stern, M. Z., Nyambega, B., Barda, O., Nasiga, D., Gupta, S. K., Michaeli, S. & Levin, M. J., 2009.** Functional characterization and protein-protein interactions of trypanosome splicing factors U2AF35, U2AF65 and SF1. *Molecular and Biochemical Parasitology*, **164** (2): 137-146.

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Early in the assembly of eukaryotes the branch-point binding protein (BBP, also called SF1) recognizes the branch point sequence, whereas the heterodimer U2AF, consisting of a 65 and a 35 kDa subunit, contacts the polypyrimidine tract and the AG splice site, respectively. Herein, we identified, cloned and expressed the *Trypanosoma cruzi* and

Trypanosoma brucei U2AF35, U2AF65 and SF1. Trypanosomatid U2AF65 strongly diverged from yeast and human homologues. On the contrary, trypanosomatid SF1 was conserved but lacked the C-terminal sequence present in the mammalian protein. Yeast two hybrid approaches were used to assess their interactions. The interaction between U2AF35 and U2AF65 was very weak or not detectable. However, as in other eukaryotes, the interaction between U2AF65 and SF1 was strong. At the cellular level, these results were confirmed by fractionation and affinity-selection experiments in which SF1 and U2AF65 were affinity-selected with TAP tagged SF1, but not with TAP tagged U2AF35. Silencing one of the three factors affected growth and trans-splicing in the first step of this reaction. Trypanosomes are the first described example of eukaryotic cells in which the interaction of two expressed U2AF factors seemed to be very weak, or not detectable.

14956. **Verplaetse, E., Rigden, D. J. & Michels, P. A., 2009.** Identification, characterization and essentiality of the unusual peroxin 13 from *Trypanosoma brucei*. *Biochimica and Biophysica Acta*, **1793** (3): 516-527.

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Peroxin 13 (PEX13) is one of the components of a peroxisomal membrane complex involved in import of proteins into the matrix of the organelles and has previously been characterized in a variety of organisms. Trypanosomatids (*Trypanosoma*, *Leishmania*), which are protozoan parasites having peroxisome-like organelles designated as glycosomes, and possess an unusual PEX13 which shares very low sequence identity with others and lacks some typical PEX13 characteristics. It was identified in the databases through its multiple YGx motifs present in a glycine-rich N-terminal region of low sequence complexity. Like other PEX13s, it contains predicted transmembrane segments and a SH3 domain in its C-terminal half. The localization of *T. brucei* PEX13 in the glycosomal membrane was confirmed by expression of a fusion construct with green fluorescent protein, and western blot analysis of purified organelles and membranes. The C-terminal half of the protein was shown to interact with the third of three pentapeptide repeats of the previously characterized PEX5, the receptor of glycosomal proteins with a type 1 peroxisome-targeting signal, and with PEX14, another component of the same peroxisomal protein import complex in the membrane. PEX13 is essential for the parasite; depletion by RNA interference results in mislocalization of glycosomal proteins and death of the parasites.

14957. **Walrad, P., Paterou, A., Acosta-Serrano, A. & Matthews, K. R., 2009.** Differential trypanosome surface coat regulation by a CCCH protein that co-associates with procyclin mRNA cis-elements. *PLoS Pathogens*, **5** (2): e1000317.

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The genome of *Trypanosoma brucei* is unusual in being regulated almost entirely at the post-transcriptional level. In terms of regulation, the best-studied genes are procyclins, which encode a family of major surface GPI-anchored glycoproteins (EP1, EP2, EP3, GPEET) that show differential expression in the parasite's tsetse fly vector. Although procyclin mRNA cis-regulatory sequences have provided the paradigm for post-

transcriptional control in kinetoplastid parasites, trans-acting regulators of procyclin mRNAs are unidentified, despite intensive effort over 15 years. Here we identify the developmental regulator, *TbZFP3*, a CCH-class predicted RNA binding protein, as an isoform-specific regulator of Procyclin surface coat expression in trypanosomes. We demonstrate (i) that endogenous *TbZFP3* shows sequence-specific co-precipitation of EP1 and GPEET, but not EP2 and EP3, procyclin mRNA isoforms, (ii) that ectopic overexpression of *TbZFP3* does not perturb the mRNA abundance of procyclin transcripts, but rather that (iii) their protein expression is regulated in an isoform-specific manner, as evidenced by mass spectrometric analysis of the Procyclin expression signature in the transgenic cell lines. The *TbZFP3* mRNA-protein complex (*TbZFP3*mRNP) is identified as a trans-regulator of differential surface protein expression in trypanosomes. Moreover, its sequence-specific interactions with procyclin mRNAs are compatible with long-established predictions for Procyclin regulation. Combined with the known association of *TbZFP3* with the translational apparatus, this study provides a long-sought missing link between surface protein cis-regulatory signals and the gene expression machinery in trypanosomes.

14958. **Weng, J., Aphasizheva, I., Etheridge, R. D., Huang, L., Wang, X., Falick, A. M. & Aphasizhev, R., 2008.** Guide RNA-binding complex from mitochondria of trypanosomatids. *Molecular Cell*, **32** (2): 198-209.

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In the mitochondria of trypanosomatids, the majority of mRNAs undergo massive uracil-insertion/deletion editing. Throughout the processes of pre-mRNA polyadenylation, guide RNA (gRNA) uridylation and annealing to mRNA, and editing reactions, several multiprotein complexes must engage in transient interactions to produce a template for protein synthesis. Here, we report the identification of a protein complex essential for gRNA stability. The gRNA-binding complex (GRBC) interacts with gRNA processing, editing, and polyadenylation machineries and with the mitochondrial edited mRNA stability (MERS1) factor. RNAi knockdown of the core subunits, GRBC1 and GRBC2, led to the elimination of gRNAs, thus inhibiting mRNA editing. Inhibition of MERS1 expression selectively abrogated edited mRNAs. Homologous proteins unique to the order of Kinetoplastida, GRBC1 and GRBC2, form a stable 200 kDa particle that directly binds gRNAs. Systematic analysis of RNA-mediated and RNA-independent interactions involving the GRBC and MERS1 suggests a unified model for RNA processing in the kinetoplast mitochondria.

14959. **Xiao, Y., McCloskey, D. E. & Phillips, M. A., 2009.** Ornithine decarboxylase and spermidine synthase RNAi-mediated gene silencing in *Trypanosoma brucei* provides insight into the regulation of polyamine biosynthesis. *Eukaryotic Cell*, **8**(5): 747-755.

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Polyamine biosynthesis is a drug target for the treatment of African sleeping sickness, however mechanisms regulating the pathway in *Trypanosoma brucei* are not well understood. Recently we showed that RNAi-mediated gene silencing or inhibition of S-adenosylmethionine decarboxylase (AdoMetDC) led to upregulation of the AdoMetDC activator, prozyme, and ornithine decarboxylase (ODC) proteins. To determine if this regulatory response is specific to AdoMetDC we studied the effects of RNAi-induced silencing of the spermidine synthase (SpdSyn) and ODC genes in bloodstream form *T. brucei*. Knockdown of either gene product led to depletion of polyamine and trypanothione pools, and to cell death. Decarboxylated AdoMet levels were elevated, while AdoMet was not affected. There was no significant effect on protein levels of other polyamine pathway enzymes. Treatment of parasites with the ODC inhibitor alpha-difluoromethylornithine (DFMO) gave similar results to those observed for ODC knockdown. Thus the cellular response to loss of AdoMetDC activity is distinctive, suggesting that AdoMetDC activity controls expression levels of the other spermidine biosynthetic enzymes. ODC RNAi-mediated cell death occurred more rapidly than for SpdSyn. Further the ODC RNAi cells were rescued by putrescine, but not spermidine, suggesting that depletion of both putrescine and spermidine is more detrimental than depletion of spermidine alone. This finding may contribute to the effectiveness of ODC as a target for the treatment of African sleeping sickness, thus providing important insight into the mechanism of action of a key anti-trypanosomal agent.

14960. **Zhaorigetu, S., Wan, G., Kaini, R., Jiang, Z. & Hu, C. A., 2008.** ApoL1, a BH3-only lipid-binding protein, induces autophagic cell death. *Autophagy*, **4** (8): 1079-1082.

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We recently reported the identification and characterization of a novel BH3-only pro-death protein, apolipoprotein L1 (ApoL1), that, when overexpressed, induces autophagic cell death (ACD) in a variety of cells, including those originated from normal and cancerous tissues. ApoL1 failed to induce ACD in autophagy-deficient Atg5(-/-) and Atg7(-/-) MEF cells, suggesting that ApoL1-induced cell death is indeed autophagy-dependent. In addition, a BH3 domain deletion allele of ApoL1 was unable to induce ACD, demonstrating that ApoL1 is a *bona fide* BH3-only pro-death protein. To further investigate regulation of ApoL1 expression, we showed that ApoL1 is inducible by interferon-gamma and tumour necrosis factor-alpha in human umbilical vein endothelial cells, suggesting that ApoL1 may play a role in cytokine-induced inflammatory response. Moreover, we observed that ApoL1 is a lipid-binding protein with high affinity for phosphatidic acid and cardiolipin and less affinity for various phosphoinositides. Functional genomics analysis identified 5 non-synonymous single nucleotide polymorphisms (NSNPs) in the coding exons of the human ApoL1 structural gene-all the 5 NSNPs may cause deleterious alteration of ApoL1 activity. Finally, we discuss the link between ApoL1 and various human diseases.

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