reach patients trapped by violence is a major challenge. Context-appropriate programme approaches that take into account the complex epidemiology of HAT and the precarious situations in which it is found are still necessary.

NECT has a number of limitations as a treatment option for HAT. It is likely less effective against *T. b. rhodesiense* HAT, which badly needs different and better drugs for both stages of the disease. Administration of NECT is relatively complicated, including the requirement of two IV infusions per day for one week. Although this protocol is shorter and simpler than eflornithine monotherapy, and safer than melarsoprol, it is still resource- and training-intensive. Thus, a simpler regimen, preferably based on an oral drug formulation, is desirable. A treatment effective for both disease stages may eliminate the need for painful lumbar punctures and difficult examination of the cerebrospinal fluid, which are currently performed for HAT staging.

R&D for better diagnostic tools for HAT are also needed. The sensitivity of parasite detection tools in body fluids is currently limited. In addition, diagnosis of trypanosomal infection of the central nervous system requires a lumbar puncture, which is painful and difficult to perform, especially in resource-constrained settings. Field-adapted, rapid diagnostic tests for HAT diagnosis and staging must be developed if complete HAT control, including integration into primary health care centers, is to be feasible. The introduction of novel biomarkers, including recently identified markers for disease staging, and the development of field-adapted tests will require the mobilization of research laboratories with adequate funding.

Although there has been recent discourse that the elimination of HAT is feasible, this lofty goal is not likely to be possible in the near future given ongoing constraints, namely the difficulties of implementing complex diagnostic–treatment algorithms in resource-poor areas of high endemicity and persistent security threats. Even if perfect treatment and diagnostic tools were readily available for HAT, certain patient populations would still be difficult or impossible to reach. HAT control in these hotspots should therefore be addressed through targeted programming and access, with robust surveillance and response. International donors and policymakers should be made aware that a "one size fits all" integrated approach may not be suitable for HAT in certain contexts and with the current tools. Dedicated funding for diagnosis and treatment and R&D, as well as allocated national programme funding, must be put forth and sustained. The current paucity of international donors funding HAT control national programmes is highly worrisome. Still and in the future, continued political pressure and will are needed for the prioritization of HAT patient care.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

15260. **Abebe, R. & Wolde, A., 2010**. A cross-sectional study of trypanosomosis and its vectors in donkeys and mules in Northwest Ethiopia. *Parasitology Research*, **106** (4): 911-916.

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A preliminary study was conducted in January 2009 in four peasant associations (PAs) selected from two districts in Benishangul Gumuz Regional State, Northwest Ethiopia to

investigate the prevalence and species of trypanosomes infecting donkeys and mules and identify the fly vectors playing a role in the transmission of trypanosomosis. Blood samples were collected from a total of 334 donkeys and 52 mules and examined by dark ground/phase contrast buffy coat technique and Giemsa-stained blood smears. Accordingly, trypanosome species were encountered in 6.3 percent of the examined donkeys (n = 21) while none of the mules examined was positive for trypanosome infection. Trypanosomes and tsetse flies were detected in two of the four PAs surveyed (Tsetsa adurno and Bamadone) with significant (P = 0.004) difference in prevalence. The inability to find trypanosomes in the other two PAs (Ura and Ashura) was most likely due to the absence of appropriate fly vectors. Three species of trypanosomes were detected in donkeys, which in order of predominance were *Trypanosoma congolense* (52.4 percent), *Trypanosoma brucei* (28.6 percent), and *Trypanosoma vivax* (19.05 percent). There was a significant (P = 0.008) difference in mean PCV between trypanosome infected and non-infected donkeys. The body condition score of the donkeys was significantly associated with both prevalence of infection (P = 0.009) and mean packed cell volume (PCV; P < 0.0001). No significant difference was observed between male and female donkeys regarding both prevalence of infection and mean PCV (P > 0.05 for each factor). The entomological surveys revealed the presence of *Glossina morsitans submorsitans* and other biting flies of the family *Stomoxys, Tabanus*, and *Haematopota*. In conclusion, the prevalence of trypanosomosis obtained in the current study is generally low compared to previous studies. As the present study design was a cross-sectional, one that only depicts a momentary picture of the infection status in the herd, a further longitudinal study that makes use of more sensitive techniques and entomological survey is recommended.

15261. **Cordon-Obras, C., Garcia-Estebanez, C., Ndong-Mabale, N., Abaga, S., Ndongo-Asumu, P., Benito, A. & Cano, J., 2010.** Screening of *Trypanosoma brucei gambiense* in domestic livestock and tsetse flies from an insular endemic focus (Luba, Equatorial Guinea). *PLoS Neglected Tropical Diseases,* **4** (6): e704.

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Sleeping sickness is spread over 36 Sub-Saharan African countries. In West and Central Africa, the disease is caused by *Trypanosoma brucei gambiense*, which produces a chronic clinical manifestation. The Luba focus (Bioko Island, Equatorial Guinea) has not reported autochthonous sleeping sickness cases since 1995, but given the complexity of the epidemiological cycle, the elimination of the parasite in the environment is difficult to categorically ensure. The aim of this work is to assess, by a molecular approach (Polymerase Chain Reaction, PCR), the possible permanence of *T. b. gambiense* in the vector (*Glossina* spp.) and domestic fauna in order to improve our understanding of the epidemiological situation of the disease in an isolated focus considered to be under control. The results obtained show the absence of the parasite in peridomestic livestock but its presence, although at very low rate, in the vector. On the other hand, interesting entomological data highlight that an elevated concentration of tsetse flies was observed in two out of the ten villages

considered to be in the focus. These findings demonstrate that even in conditions of apparent control, a complete parasite clearance is difficult to achieve. Further investigations must be focused on animal reservoirs which could allow the parasites to persist without leading to human cases. In Luba, where domestic livestock are scarcer than other foci in mainland Equatorial Guinea, the epidemiological significance of wild fauna should be assessed to establish their role in the maintenance of the infection.

15262. **Efrem, D. B., Yacob, H. T., Hagos, A. T. & Basu, A. K., 2010**. Bovine trypanosomosis in Gimbi district of Western Oromia, Ethiopia. *Animal Biology*, **60**, (2): 123–131

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A study on the epidemiology of bovine trypanosomosis was conducted from September 2006 to April 2007 in six villages of the Gimbi district in west Wollega zone of Ethiopia. The prevalence of the disease, the apparent densities and distribution of tsetse and other biting flies in two seasons, the dry and rainy, were determined. The results of a questionnaire survey from 80 farmers revealed that trypanosomosis was a major health problem affecting animals and impeding agricultural activities. A total of 568 blood samples were collected from randomly selected animals (280 animals in rainy and 288 in dry season) and revealed the presence of *Trypanosoma congolense* Broden, 1904 and *T. vivax* Zieman, 1905 in the area. *Trypanosoma congolense* was the dominant species that accounted for 66.2 percent of the infections. The mean packed cell volume (PCV) concentrations were 22.77 percent (95 percent CI =19.99-21.55) in parasitaemic and 25.25 percent (95 percent CI=24.88-25.61) in aparasitaemic animals with a significant difference ($P < 0.005$). There was a significant ($P < 0.012$) difference in trypanosome infection between age groups of cattle, being higher in adults. The overall prevalence of trypanosomosis was 12.5 percent, while the disease prevalence was higher during the rainy season (15 percent) than the dry season (10.1 percent). In three villages in lowland areas (below 1 600 meter above sea level), a higher prevalence was recorded in the late rainy and dry season respectively. (20.9 percent and 7.9 percent) as compared with 11.8 percent and 8.3 percent in three villages in midland areas (≥ 1 600 meter above sea level). A fly survey was conducted using 80 monoconical pyramidal traps and revealed that two tsetse species, namely *Glossina morsitans sub morsitans* Newstead and *Glossina tachinoides* Westwood were found along with other biting flies (*Tabanus, Haematopota* and *Stomoxys* species). Higher numerical catches of *Glossina* were recorded in the late rainy season and the apparent density was positively correlated ($r = 0.5171$) with the prevalence of infection.

15263. **Gari, F. R., Ashenafi, H., Tola, A., Goddeeris, B. M. & Claes, F., 2010.** Comparative diagnosis of parasitological, serological, and molecular tests in dourine-suspected horses. *Tropical Animal Health & Production.* **Published online 6 June**.

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A study on the comparative sensitivity of parasitological, serological, and molecular tests on 237 horses originating from two dourine-suspected districts of Arsi-Bale highlands of Ethiopia was conducted to determine the prevalence of the disease and degree of agreement of the diagnostic tests. Accordingly, the prevalence of the disease was found to be 4.6 percent, 36.7 percent, and 47.6 percent by parasitological Woo test, RoTat 1.2 and 18S PCR tests, respectively. The seroprevalence of the disease was 27.6 percent in CATT/*Trypanosoma evansi* test. In Ethiopia, it was for the first time that trypanosomes from dourine suspected horses were demonstrated in 4.6 percent of the animals using Woo test. The findings of the present study disclosed that dourine is highly prevalent and one of the major diseases of horses in the area. There was no statistically significant difference ($P > 0.05$) in prevalence of the disease between districts, sexes, and age groups of the animals. However, there was a statistically significant difference ($P < 0.05$) in the prevalence of the disease between emaciated and animals with good body condition. Assessment of the degree of agreement of the diagnostic tests employed revealed low to fair with significantly higher sensitivity by PCR than other tests.

15264. **Munang'andu, H. M., Siamudaala, V., Munyeme, M., Nambota, A., Mutoloki, S. & Matandiko, W., 2010**.*Trypanosoma brucei* infection in asymptomatic greater Kudus (*Tragelaphus strepsiceros*) on a game ranch in Zambia. *Korean Journal of Parasitology,* **48** (1): 67-69.

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Trypomastogotes of *Trypanosoma brucei* were detected from 4 asymptomatic kudus (*Tragelaphus strepsiceros*) on a game ranch located approximately 45 km north east of Lusaka, Zambia. Blood smears examined from 14 wildlife species comprising of the impala (*Aepyceros melampus*), kafue lechwe (*Kobus leche kafuensis*), sable antelope (*Hippotragus niger*), tsessebe (*Damaliscus lunatus*), warthog (*Phacochoerus aethiopicus*), puku (*Kobus vardoni*), zebra (*Equus burchelli*), waterbuck (*Kobus ellipsiprymnus*), bushbuck (*Tragelaphus scriptus*), reedbuck (*Redunca arundinum*), wildebeest (*Connochaetes taurinus*), hartebeest (*Alcephelus lichtensteini*), African buffalo (*Syncerus caffer*), and kudu (*Tragelaphus strepsiceros*) showed that only the kudu had *T. brucei*. Although game ranching has emerged to be a successful *ex situ* conservation strategy aimed at saving the declining wildlife population in the national parks, our findings suggest that it has the potential of aiding the re-distribution of animal diseases. Hence, there is a need for augmenting wildlife conservation with disease control strategies aimed at reducing the risk of disease transmission between wildlife and domestic animals.

15265. **Simukoko, H., Marcotty, T., Vercruysse, J. & Van den Bossche, P., 2010.** Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia. *Research in Veterinary Science.* **In press, corrected proof.**

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The control of bovine trypanosomiasis could be improved by using the available control tools during periods when the incidence of the disease is highest. The present study assessed the monthly risk of bovine trypanosomiasis in 85 sentinel cattle kept on the tsetse-infested eastern plateau of Zambia during a period of 19 consecutive months. To avoid problems associated with persistence of infections because of trypanocidal drug resistance and/or the time lag between sampling and molecular analysis, a survival analysis and the subsequent calculation of risk was used as an indicator of challenge. Results showed that the average monthly risk of infection (92.3 percent due to *Trypanosoma congolense*) was 6 percent. It was significantly higher (7.7 percent) during the beginning of the rainy season (December-February). According to the outcome of the study, bovine trypanosomiasis control in the study area can be improved through increasing control efforts during this period of highest challenge.

15266. **Tadesse, A. & Tsegaye, B., 2010.** Bovine trypanosomosis and its vectors in two districts of Bench Maji zone, South Western Ethiopia. *Tropical Animal Health & Production.* **Published online 26 June.**

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A cross-sectional study was carried out from November 2008 to February 2009 in Guraferda and Sheko districts of Bench Maji Zone, South Western Ethiopia. The objective of the study was to determine the prevalence of bovine trypanosomosis and the density of its vectors. An overall prevalence of trypanosome infection in the study area was 4.4 percent. *Trypanosoma congolense* (36.36 percent) was the dominant trypanosome species followed by *Trypanosoma vivax* (18.18 percent) and *Trypanosoma brucei* (9.09 percent). Mean packed cell volume value of parasitaemic animals (21.8 percent) was significantly ($P < 0.05$) lower than that of aparasitaemic animals (27.7 percent). Biconical and NGU traps were deployed for 72 h, and the result indicated *Glossina pallidipes* followed by *Glossina fuscipes* as the only tsetse fly species caught in the study area along with other biting flies like *Stomoxys* and *Tabanus*. The apparent density of tsetse flies was 2.83 flies $trap^{-1}$ day^{-1}. NGU traps caught more of *G. pallidipes* while biconical traps caught more *G. fuscipes*, and the difference was significant ($P < 0.05$). Although the current study indicated low prevalence of trypanosomosis in the study area, the impacts of trypanosomosis on cattle production and productivity should not be neglected. Therefore, attention should be given to control the disease and also the vectors.

15267. **Thumbi, S. M., Jung'a, J. O., Mosi, R. O. & McOdimba, F. A., 2010**. Spatial distribution of African animal trypanosomiasis in Suba and Teso districts in Western Kenya. *BMC Research Notes,* **3**: 6.

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Studies on the epidemiology of African Animal Trypanosomiasis (AAT) rarely consider the spatial dimension of disease prevalence. This problem is confounded by use of parasitological diagnostic methods of low sensitivity in field surveys. Here we report a study combining highly sensitive and species specific molecular diagnostic methods, and geographical information system (GIS) for spatial analysis of trypanosome infection patterns, to better understand its epidemiology. Blood samples from 44 and 59 animals randomly selected from Teso and Suba districts respectively were screened for trypanosomes using PCR diagnostic assays. Spatial distribution of the positive cases was mapped and average nearest neighbour analysis used to determine the spatial pattern of trypanosome cases detected. Trypanosome prevalence of 41 percent and 29 percent in Suba and Teso districts respectively was observed. *T. vivax* infections were most prevalent in both areas. Higher proportions of *T. brucei* infections (12 percent) were observed in Suba, a known sleeping sickness focus compared with 2 percent in Teso. Average nearest neighbour analysis showed the pattern of trypanosome infections as random. An overlay with tsetse maps showed cases lying outside the tsetse infested areas, mostly being cases of *T. vivax* which is known to be transmitted both biologically by tsetse and mechanically by biting flies. These findings suggest a need to design control strategies that target not just the biological vector tsetse, but also the parasite in cattle in order to clear the possibly mechanically transmitted *T. vivax* infections. There is need to also review the accuracy of available tsetse maps.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **33**: 15260, 15262, 15266].

15268**. Da Silva, A. S., Wolkmer, P., Costa, M. M., Tonin, A. A., Eilers, T. L., Gressler, L. T., Otto, M. A., Zanette, R. A., Santurio, J. M., Lopes, S. T. & Monteiro, S. G., 2010.** Biochemical changes in cats infected with *Trypanosoma evansi*. *Veterinary Parasitology,* **171** (1-2): 48-52.

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This study aimed at evaluating biochemical changes of cats (*Felis catus*) experimentally infected with *Trypanosoma evansi*. Seven animals were infected with 10^8 blood trypomastigotes per animal and six were used as controls. Blood smears were performed daily for 56 days and the hepatic, renal and muscular parameters in blood serum were evaluated at days 0, 7, 21, 35 and 49. The protozoan was found in the bloodstream 24-48 h post-inoculation (PI) and irregular peaks of parasitaemia were observed throughout the experiment. Muscular enzymatic activities (aspartate aminotransferase and creatine kinase)

were increased in infected cats compared to controls. Increased concentrations of total proteins and globulins and decreased levels of albumin and albumin/globulin ratio were observed in infected group versus the controls values ($P < 0.05$). No alteration in serum activity of alanine aminotransferase, gamma-glutamyltransferase, creatinine and urea was observed in both groups.

(c) TRYPANOTOLERANCE

[See also **33:** 15291, 15293, 15294, 15371].

15269. **Behnke, J. M., Chiejina, S. N., Musongong, G. A., Nnadi, P. A., Ngongeh, L. A., Abonyi, F. O. & Fakae, B. B., 2010.** Resistance and resilience of traditionally managed West African Dwarf goats from the savannah zone of northern Nigeria to naturally acquired trypanosome and gastrointestinal nematode infections. *Journal of Helminthology.* **e publication May 12.**

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A survey was conducted of gastrointestinal nematode infections and trypanosomosis in Nigerian West African Dwarf (WAD) goats from the savannah region of the country. Animals were screened at two markets, Gboko and Akpagher, from the beginning of April until the end of September, coinciding with the end of the dry season and the first 5 months of the wet season. Of 1 054 goats that were examined, 80.5 percent carried gastrointestinal (GI) nematodes belonging to the genera *Haemonchus* (61.0 percent), *Oesophagostomum* (21.0 percent) and *Trichostrongylus* (17.9 percent). Faecal egg counts (FEC) increased very slowly but significantly from April to maximum levels in September, and varied marginally between the two market sources. The majority of goats (68.8 and 70.1 percent at the two markets) had low FEC not exceeding 50 eggs/g (epg). FEC did not differ significantly between the sexes or between age classes. Packed cell volume (PCV) also declined significantly with month of the study, but was affected by host sex (a significant month x sex interaction) being generally higher in male animals throughout the period. There was a highly significant negative correlation between $\log_{10}$ (FEC+1) and PCV, when all other factors had been taken into account. Body condition scores (BCS) also declined with month of the study, but there was a marked difference between the two sexes, with male animals generally showing a greater stability of BCS across the months compared with females. Trypanosome infections were found in only 4 percent of the goats and only during the rainy season. Most infections (92.86 percent) were caused by *Trypanosoma brucei* alone although *T. vivax* and *T. congolense* were occasionally detected. Overall, the majority of goats sampled each month maintained generally good body condition (BCS 3.0-5.0), normal or slightly reduced PCV, even when concurrently infected with trypanosomes and GI nematodes. However, four concurrently infected goats showed signs of overt anaemia during periods of peak infection, during the late rainy season, with marked reductions in PCV (<15 percent). Two of the infected goats were also in poor body condition with BCS of < 2.0. There was no evidence of additive or synergistic pathogenic effects of the two parasites. These results are discussed in

the context of the unexpectedly strong resistance and resilience of the savannah WAD ecotype to its native strains of GI nematode and trypanosome parasites.

(d) TREATMENT

[See **33**: 15311].

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

[See also **33**: 15198, 15203, 15205, 15263].

15270. **Camara, M., Camara, O., Ilboudo, H., Sakande, H., Kabore, J., N'Dri, L., Jamonneau, V. & Bucheton, B., 2010**. Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test. *Tropical Medicine & International Health,* **15** (7): 796-799.

Programme National de Lutte contre la Trypanosomose Humaine Africaine, Conakry, Guinea.

This study was to evaluate a modification of the mini anion exchange centrifugation test (mAECT) for the diagnosis of *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT). To increase its sensitivity, this test uses 350 µL of buffy coat withdrawn from 5 mL of blood instead of blood. The new protocol was first tested experimentally on serial dilution of trypanosomes and was then further evaluated under field conditions on 57 patients with HAT diagnosed during a medical survey in Guinea. Experimentally, the use of buffy coats improved mAECT sensitivity at least five fold and enabled to consistently detect parasites in blood at a concentration of 10 trypanosomes/mL. During the field evaluation, more patients tested positive by mAECT-bc (96.5 percent) than by mAECT-blood (78.9 percent, $chi^2 = 6.93$, $P = 0.008$) and lymph juice examination (77.2 percent, $chi^2 = 7.67$, $P = 0.005$). Furthermore, the number of parasites per collectors was significantly higher (7.2 vs. 2.6, $P = 0.001$) when buffy coats were used instead of blood. The use of the mAECT-bc protocol enabled a significant improvement of HAT parasitological diagnosis in Guinea, without any additional costs. It would deserve to be tested in other *T. b. gambiense* endemic areas.

15271. **de Clare Bronsvoort, B. M., von Wissmann, B., Fevre, E. M., Handel, I. G., Picozzi, K. & Welburn, S. C., 2010.** No gold standard estimation of the sensitivity and specificity of two molecular diagnostic protocols for *Trypanosoma brucei* spp. in Western Kenya. *PLoS One*, **5** (1): e8628.

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African animal trypanosomiasis is caused by a range of tsetse transmitted protozoan parasites including *Trypanosoma vivax, Trypanosoma congolense* and *Trypansoma brucei*. In Western Kenya and other parts of East Africa two subspecies of *T. brucei, T. b. brucei* and the zoonotic *T. b. rhodesiense*, co-circulate in livestock. A range of polymerase chain reactions (PCR) have been developed as important molecular diagnostic tools for epidemiological investigations of *T. brucei* s.l. in the animal reservoir and of its zoonotic potential. Quantification of the relative performance of different diagnostic PCRs is essential to ensure comparability of studies. This paper describes an evaluation of two diagnostic test systems for *T. brucei* using a *T. brucei* s.l. specific PCR and a single nested PCR targeting the internal transcribed spacer (ITS) regions of trypanosome ribosomal DNA. A Bayesian formulation of the Hui-Walter latent class model was employed to estimate their test performance in the absence of a gold standard test for detecting *T. brucei* s.l. infections in ear-vein blood samples from cattle, pig, sheep and goat populations in Western Kenya, stored on Whatman FTA cards. The results indicate that the system employing the *T. brucei* s.l. specific PCR (Se1 = 0.760) had a higher sensitivity than the ITS-PCR (Se2 = 0.640); both have high specificity (Sp1 = 0.998; Sp2 = 0.997). The true prevalences for livestock populations were estimated (pcattle=0.091, ppigs = 0.066, pgoats = 0.005, psheep = 0.006), taking into account the uncertainties in the specificity and sensitivity of the two test systems. Implications of test performance include the required survey sample size; due to its higher sensitivity and specificity, the *T. brucei* s.l. specific PCR requires a consistently smaller sample size than the ITS-PCR for the detection of *T. brucei* s.l. However the ITS-PCR is able to simultaneously screen samples for other pathogenic trypanosomes and may thus be, overall, a better choice of test in multi-organism studies.

15272. **Manful, T., Mulindwa, J., Frank, F. M., Clayton, C. E. & Matovu, E., 2010.** A search for *Trypanosoma brucei rhodesiense* diagnostic antigens by proteomic screening and targeted cloning. *PLoS One,* **5** (3): e9630.

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The only available diagnostic method for East African trypanosomiasis is light microscopy of blood samples. A simple immunodiagnostic would greatly aid trypanosomiasis control. To find trypanosome proteins that are specifically recognized by sera from human sleeping sickness patients, we have screened the *Trypanosoma brucei brucei* proteome by Western blotting. Using cytosolic, cytoskeletal and glycosomal fractions, we found that the vast majority of abundant trypanosome proteins are not specifically recognized by patient sera. We identified phosphoglycerate kinase (PGKC), heat shock protein (HSP70), and histones H2B and H3 as possible candidate diagnostic antigens. These proteins, plus paraflagellar rod protein 1, rhodesain (a cysteine protease), and an extracellular fragment of the *Trypanosoma brucei* nucleoside transporter TbNT10, were expressed in *E. coli* and tested for reactivity with patient and control sera. Only TbHSP70 was preferentially recognized by

patient sera, but the sensitivity and specificity were insufficient for use of TbHSP70 alone as a diagnostic. Immunoprecipitation using a native protein extract revealed no specifically reacting proteins. It is concluded that no abundant *T. brucei* soluble, glycosomal or cytoskeletal protein is likely to be useful in diagnosis. To find useful diagnostic antigens it will therefore be necessary to use more sophisticated proteomic methods, or to test a very large panel of candidate proteins.

15273**. Mugasa, C. M., Deborggraeve, S., Schoone, G. J., Laurent, T., Leeflang, M. M., Ekangu, R. A., El Safi, S., Saad, A. F., Basiye, F. L., De Doncker, S., Lubega, G. W., Kager, P. A., Buscher, P. & Schallig, H. D., 2010**. Accordance and concordance of PCR and NASBA followed by oligochromatography for the molecular diagnosis of *Trypanosoma brucei* and *Leishmania*. *Tropical Medicine & International Health,* **15** (7): 800-805.

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To evaluate the repeatability and reproducibility of four simplified molecular assays for the diagnosis of *Trypanosoma brucei* spp. or *Leishmania* ssp. in a multicentre ring trial with seven participating laboratories, samples were tested using PCR or NASBA amplification of the parasites' nucleic acids followed by rapid read-out by oligochromatographic dipstick (PCR-OC and NASBA-OC). On purified nucleic acid specimens, the repeatability and reproducibility of the tests were Tryp-PRC-OC, 91.7 percent and 95.5 percent; Tryp-NASBA-OC, 95.8 percent and 100 percent; Leish-PCR-OC, 95.9 percent and 98.1 percent; Leish-NASBA-OC, 92.3 percent and 98.2 percent. On blood specimens spiked with parasites, the repeatability and reproducibility of the tests were Tryp-PRC-OC, 78.4 percent and 86.6 percent; Tryp-NASBA-OC, 81.5 percent and 89.0 percent; Leish-PCR-OC, 87.1 percent and 91.7 percent; Leish-NASBA-OC, 74.8 percent and 86.2 percent. As repeatability and reproducibility of the tests were satisfactory, further phase II and III evaluations in clinical and population specimens from disease endemic countries are justified.

15274. **Sengupta, P. P., Balumahendiran, M., Suryanaryana, V. V., Raghavendra, A. G., Shome, B. R., Gajendragad, M. R. & Prabhudas, K., 2010**. PCR-based diagnosis of surra-targeting VSG gene: experimental studies in small laboratory rodents and buffalo. *Veterinary Parasitology*, **171** (1-2): 22-31.

Project Directorate on Animal Disease Monitoring and Surveillance, Hebbal, Bangalore 560024, Karnataka, India. [pinakiprasad_s@rediffmail.com].

Trypanosoma evansi, the causative organism of "surra" expresses its variable surface glycoprotein (VSG) at early, middle and late stages of infection in animals. The variable antigenic nature of VSG caused by switching its expression type favours evasion from the host immune response and leads to chronic and persistent infection. Developing a polymerase chain reaction (PCR)-based diagnostic tool targeting the VSG gene is expected to be highly specific and sensitive for diagnosis of surra. Hence, in the present study, we have designed EXP3F/4R primer pair and amplified the 1.4 kb of VSG gene of *T. evansi* and studied the phylogenetic relationship by *in silico* analysis. The PCR method was standardized using another set of primers, DITRYF/R, and 400 bp were amplified from blood and tissue samples of experimentally infected animals. Applying the PCR method, we were able to detect as low as 0.15 trypanosomes/mL^{-1}. Considering the number of parasites and DNA concentrations, the PCR method has a sensitivity of 0.015 pg/mL^{-1}. The PCR could detect the presence of the parasite as early as 24h post-infection (p.i.) and 72 h p.i., respectively, in experimentally infected rats and buffalo. No amplification was observed with DNA of *Babesia bigemina* and *Theileria annulata*, indicating the primers are specific for *T. evansi*. The PCR method could detect the dog, lion and leopard isolates of *T. evansi*. Similarly, amplifying the DNA from the experimentally infected tissues was also found to be sensitive. Thus, the findings of this study favour the application of PCR over the parasitological methods for the detection of the early and/or chronic stage of surra in domestic and wild animals.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **33**: 15371, 15399, 15401].

15275. **Amin, D. N., Ngoyi, D. M., Nhkwachi, G. M., Palomba, M., Rottenberg, M., Buscher, P., Kristensson, K. & Masocha, W., 2010**. Identification of stage biomarkers for human African trypanosomiasis. *American Journal of Tropical Medicine & Hygiene*, **82** (6): 983-990.

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Human African trypanosomiasis (HAT), caused by infection with sub-species of *Trypanosoma brucei* (*T. b.*), manifests as a haemolymphatic stage followed by an encephalitic stage. The distinction of the two stages needs improvement as drugs used for the late stage are highly toxic. Transcripts encoding 16 secreted proteins differentially expressed in the brains of mice at late stage *T. b. brucei* infection when the early stage drug suramin is no longer effective and different to immunoglobulins, chemokines, and cytokines, were selected by microarray analysis. Lipocalin 2 and secretory leukocyte peptidase inhibitor (SLPI) mRNA showed the highest differential expression in mice. These transcripts were also upregulated in brains from infected rats. Lipocalin 2 was increased in cerebrospinal fluid

(CSF) from rats during late stage *T. b. brucei* infection. Protein levels of lipocalin 2, SLPI, and the chemokine CXCL10 were found increased in CSF from *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* late stage HAT compared to early stage.

15276**. Amrouni, D., Gautier-Sauvigne, S., Meiller, A., Vincendeau, P., Bouteille, B., Buguet, A. & Cespuglio, R., 2010.** Cerebral and peripheral changes occurring in nitric oxide (NO) synthesis in a rat model of sleeping sickness: identification of brain iNOS expressing cells. *PLoS One,* **5** (2): e9211.

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The implication of nitric oxide (NO) in the development of human African trypanosomiasis (HAT) using an animal model, was examined. The manner by which the trypanocidal activity of NO is impaired in the periphery and in the brain of rats infected with *Trypanosoma brucei brucei* (*T. b. brucei*) was analyzed through: (i) the changes occurring in NO concentration in both peripheral (blood) and cerebral compartments; (ii) the activity of nNOS and iNOS enzymes; (iii) identification of the brain cell types in which the NO-pathways are particularly active during the time-course of the infection. NO concentration (direct measures by voltammetry) was determined in central (brain) and peripheral (blood) compartments in healthy and infected animals at various days post-infection: D5, D10, D16 and D22. Opposite changes were observed in the two compartments. NO production increased in the brain (hypothalamus) from D10 (+32 percent) to D16 (+71 percent), but decreased in the blood from D10 (-22 percent) to D16 (-46 percent) and D22 (-60 percent). In parallel with NO measures, cerebral iNOS activity increased and peaked significantly at D16 (up to +700 percent). However, nNOS activity did not vary. Immunohistochemical staining confirmed iNOS activation in several brain regions, particularly in the hypothalamus. In peritoneal macrophages, iNOS activity decreased from D10 (-83 percent) to D16 (-65 percent) and D22 (-74 percent) similarly to circulating NO. The NO changes observed in our rat model were dependent on iNOS activity in both peripheral and central compartments. In the periphery, the NO production decrease may reflect an arginase-mediated synthesis of polyamines necessary to trypanosome growth. In the brain, the increased NO concentration may result from an enhanced activity of iNOS present in neurons and glial cells. It may be regarded as a marker of deleterious inflammatory reactions.

15277. **Vankrunkelsven, A., De Ceulaer, K., Hsu, D., Liu, F-T., De Baetselier, P. & Stiljemans, B., 2010.** Lack of galactin-3 alleviateds trypanosomiasis-associated anaemia of inflammation. *Immunobiology,* **215** (9-10): 833-841.

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A typical pathological feature associated with experimental African trypanosomiasis (*Trypanosoma brucei* infection in mice) is anaemia of chronic disease (ACD), which is due to a sustained type 1 cytokine-mediated inflammation and hyperactivation of M1 macrophages. Galectin-3 (Gal-3) was amply documented to contribute to the onset and persistence of type 1 inflammatory responses and we herein document that this protein is strongly upregulated during *T. brucei* infection. We evaluated the involvement of Gal-3 in trypanosomiasis-associated anaemia using galectin-3 deficient (Gal3(-/-)) mice. *T. brucei* infected Gal3(-/-) mice manifested significant lower levels of anaemia during infection and survived twice as long as wild type mice. Moreover, such mice showed increased levels of serum IL-10 and reduced liver pathology (as evidenced by lower AST/ALT levels). In addition, there was also an increase in gene expression of iron export genes and a reduced expression of genes, which are associated with accumulation of cellular iron. Our data indicate that Gal-3 is involved in the development of inflammation-associated anaemia during African trypanosomiasis, possibly due to a disturbed iron metabolism that in turn may also lead to liver malfunction.

15278. **Bastos, I. M., Motta, F. N., Charneau, S., Santana, J. M., Dubost, L., Augustyns, K. & Grellier, P., 2010.** Prolyl oligopeptidase of *Trypanosoma brucei* hydrolyzes native collagen, peptide hormones and is active in the plasma of infected mice. *Microbes & Infection,* **12** (6): 457-466.

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Proteases play important roles in many biological processes of parasites, including their host interactions. In sleeping sickness, *Trypanosoma brucei* proteases released into the host bloodstream could hydrolyze host factors, such as hormones, contributing to the development of the disease's symptoms. In this study, we present the identification of the *T. brucei* prolyl oligopeptidase gene (poptb) and the characterization of its corresponding enzyme, POP Tb. Secondary structure predictions of POP Tb show a structural composition highly similar to other POPs. Recombinant POP Tb produced in *E. coli* was active and highly sensitive to inhibitors of *Trypanosoma cruzi* POP Tc80. These inhibitors, which prevent *T. cruzi* entry into non-phagocytic cells, arrested growth of the *T. brucei* bloodstream form in a dose-dependent manner. POP Tb hydrolyzes peptide hormones containing Pro or Ala at the P1 position at a slightly alkaline pH, and also cleaves type I collagen *in vitro* and native collagen present in rat mesentery. Furthermore, POP Tb is released into the bloodstream of *T. brucei* infected mice where it remains active. These data suggest that POP Tb might contribute to the pathogenesis of sleeping sickness.

15279. **Bocedi, A., Dawood, K. F., Fabrini, R., Federici, G., Gradoni, L., Pedersen, J. Z. & Ricci, G., 2010.** Trypanothione efficiently intercepts nitric oxide as a harmless iron complex in trypanosomatid parasites. The *FASEB Journal,* **24** (4): 1035-1042.

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Trypanosomatids are protozoan organisms that cause serious diseases, including African sleeping sickness, Chagas disease, and leishmaniasis, affecting about 30 million people in the world. These parasites contain the unusual dithiol trypanothione [T(SH)(2)] instead of glutathione (GSH) as the main intracellular reductant, and they have replaced the otherwise ubiquitous GSH/glutathione reductase redox couple with a T(SH)(2)/trypanothione reductase (TR) system. The reason for the existence of T(SH)(2) in parasitic organisms has remained an enigma. Here, we show that T(SH)(2) is able to intercept nitric oxide and labile iron and form a dinitrosyl-iron complex with at least 600 times higher affinity than GSH. Accumulation of the paramagnetic dinitrosyl-trypanothionyl iron complex *in vivo* was observed in *Trypanosoma brucei* and *Leishmania infantum* exposed to nitric oxide. While the analogous dinitrosyl-diglutathionyl iron complex formed in mammalian cells is a potent irreversible inhibitor of glutathione reductase (IC_{50} = 4 μM), the T(SH)(2) complex does not inactivate TR even at millimolar levels. The peculiar capacity of T(SH)(2) to sequester NO and iron in a harmless stable complex could explain the predominance of this thiol in parasites regularly exposed to NO.

15280. **Costa, M. M., Silva, A. S., Wolkmer, P., Zanette, R. A., Franca, R. T., Monteiro, S. G. & Lopes, S. T., 2010.** Serum proteinogram of cats experimentally infected by *Trypanosoma evansi*. *Preventive Veterinary Medicine,* **95** (3-4) 301-304.

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This study was aimed at evaluating the electrophoretic profile of serum proteins in *Trypanosoma evansi*-infected cats during different periods of infection. Thirteen adult non-breeding female *Felis catus* were separated into two groups. Animals from the infected group (n=7) were inoculated intraperitoneally with a strain of *T. evansi*; whereas, animals from the control group (n=6) received a physiological solution. Blood samples were collected at days 0, 7, 21, and 35 for total protein evaluation and protein fractionation by electrophoresis. Albumin ($P < 0.01$), alpha-2 globulin and gamma globulin ($P < 0.05$) concentrations were statistically different from the seventh day post-inoculation onwards. Beta-globulin levels were increased from day 21 onwards ($P < 0.05$). Alpha-1 globulin fraction did not differ statistically. These results indicate that the infection by *T. evansi* in cats alters the serum protein electrophoretic profile.

15281**. Das, P., Lahiri, A., Lahiri, A. & Chakravortty, D., 2010.** Modulation of the arginase pathway in the context of microbial pathogenesis: a metabolic enzyme moonlighting as an immune modulator. *PLoS Pathogens,* **6** (6): e1000899.

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Arginine is a crucial amino acid that serves to modulate the cellular immune response during infection. Arginine is also a common substrate for both inducible nitric oxide synthase (iNOS) and arginase. The generation of nitric oxide from arginine is responsible for efficient immune response and cytotoxicity of host cells to kill the invading pathogens. On the other hand, the conversion of arginine to ornithine and urea via the arginase pathway can support the growth of bacterial and parasitic pathogens. The competition between iNOS and arginase for arginine can thus contribute to the outcome of several parasitic and bacterial infections. There are two isoforms of vertebrate arginase, both of which catalyze the conversion of arginine to ornithine and urea, but they differ with regard to tissue distribution and subcellular localization. In the case of infection with Mycobacterium*, Leishmania, Trypanosoma, Helicobacter, Schistosoma*, and *Salmonella* spp., arginase isoforms have been shown to modulate the pathology of infection by various means. Despite the existence of a considerable body of evidence about mammalian arginine metabolism and its role in immunology, the critical choice to divert the host arginine pool by pathogenic organisms as a survival strategy is still a mystery in infection biology.

15282**. Emmer, B. T., Daniels, M. D., Taylor, J. M., Epting, C. L. & Engman, D. M., 2010**. Calflagin inhibition prolongs host survival and suppresses parasitaemia in *Trypanosoma brucei* infection. *Eukaryotic Cell,* **9** (6): 934-942.

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African trypanosomes express a family of dually acylated, EF-hand calcium-binding proteins called the calflagins. These proteins associate with lipid raft microdomains in the flagellar membrane, where they putatively function as calcium signalling proteins. Here we show that these proteins bind calcium with high affinity and that their expression is regulated during the life cycle stage of the parasite, with protein levels approximately 10-fold higher in the mammalian bloodstream form than in the insect vector procyclic stage. We also demonstrate a role for the calflagins in mammalian infection, as inhibition of the entire calflagin family by RNA interference dramatically increased host survival and attenuated parasitaemia in a mouse model of sleeping sickness. In contrast to infection with parental wild-type parasites, which demonstrated an unremitting parasitaemia and death within 6 to 10 days, infection with calflagin-depleted parasites demonstrated prolonged survival associated with a sudden decrease in parasitaemia at approximately 8 days postinfection. Subsequent relapsing and remitting waves of parasitaemia thereafter were associated with alternate expression of the variant surface glycoprotein, suggesting that initial clearance was antigen specific. Interestingly, despite the notable *in vivo* phenotype and flagellar localization of the calflagins, *in vitro* analysis of the calflagin-deficient parasites demonstrated normal proliferation, flagellar motility, and morphology. Further analysis of the kinetics of surface antibody clearance also did not demonstrate a deficit in the calflagin-deficient parasites; thus, the molecular basis for the altered course of infection is independent of an effect on parasite cell cycle progression, motility, or degradation of surface-bound antibodies.

15283. **Geiger, A., Hirtz, C., Becue, T., Bellard, E., Centeno, D., Gargani, D., Rossignol, M., Cuny, G. & Peltier, J. B., 2010.** Exocytosis and protein secretion in *Trypanosoma*. *BMC Microbiology,* **10**: 20.

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Human African trypanosomiasis is a lethal disease caused by the extracellular parasite *Trypanosoma brucei*. The proteins secreted by *T. brucei* inhibit the maturation of dendritic cells and their ability to induce lymphocytic allogenic responses. To better understand the pathogenic process, we combined different approaches to characterize these secreted proteins. Overall, 444 proteins were identified using mass spectrometry, the largest parasite secretome described to date. Functional analysis of these proteins revealed a strong bias toward folding and degradation processes and to a lesser extent toward nucleotide metabolism. These features were shared by different strains of *T. brucei*, but distinguished the secretome from published *T. brucei* whole proteome or glycosome. In addition, several proteins had not been previously described in *Trypanosoma* and some constitute novel potential therapeutic targets or diagnostic markers. Interestingly, a high proportion of these secreted proteins are known to have alternative roles once secreted. Furthermore, bioinformatic analysis showed that a significant proportion of proteins in the secretome lack transit peptide and are probably not secreted through the classical sorting pathway. Membrane vesicles from secretion buffer and infested rat serum were purified on sucrose gradient and electron microscopy pictures have shown 50- to 100-nm vesicles budding from the coated plasma membrane. Mass spectrometry confirmed the presence of *Trypanosoma* proteins in these microvesicles, showing that an active exocytosis might occur beyond the flagellar pocket. This study brings out several unexpected features of the secreted proteins and opens novel perspectives concerning the survival strategy of *Trypanosoma* as well as possible ways to control the disease. In addition, concordant lines of evidence support the original hypothesis of the involvement of microvesicle-like bodies in the survival strategy allowing *Trypanosoma* to exchange proteins at least between parasites and/or to manipulate the host immune system.

15284. **Harrington, J. M., Widener, J., Stephens, N., Johnson, T., Francia, M., Capewell, P., Macleod, A. & Hajduk, S. L., 2010.** The plasma membrane of bloodstream form African trypanosomes confers susceptibility and specificity to killing by hydrophobic peptides. *Journal of Biological Chemistry.* **In press, corrected proof July 13.**

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Trypanosoma brucei is the causative agent of both a veterinary wasting disease and human African trypanosomiasis, or sleeping sickness. The cellular membrane of the developmental stage found within the mammalian host, the bloodstream form (BSF), is

highly dynamic, exhibiting rapid rates of endocytosis and lateral flow of GPI-anchored proteins. Here we show that the cell membrane of these organisms is a target for killing by small hydrophobic peptides that increase the rigidity of lipid bilayers. Specifically, we have derived trypanocidal peptides that are based upon the hydrophobic N-terminal signal sequences of human apolipoproteins. These peptides selectively partition into the plasma membrane of BSF trypanosomes resulting in an increase in the rigidity of the bilayer, dramatic changes in cell motility and subsequent cell death. No killing of the developmental stage found within the insect midgut, the procyclic form, was observed. Additionally the peptides exhibit no toxicity towards mammalian cell lines nor do they induce hemolysis. Studies with model liposomes indicate that bilayer fluidity dictates the susceptibility of membranes to manipulation by hydrophobic peptides. We suggest that the composition of the BSF trypanosome cell membrane confers a high degree of fluidity and unique susceptibility to killing by hydrophobic peptides and is therefore a target for the development of trypanocidal drugs.

15285. **Inverso, J. A., Uphoff, T. S., Johnson, S. C., Paulnock, D. M. & Mansfield, J. M., 2010.** Biological variation among African trypanosomes: I. Clonal expression of virulence is not linked to the variant surface glycoprotein or the variant surface glycoprotein gene telomeric expression site. *DNA Cell Biology,* **29** (5): 215-227.

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The potential association of variant surface glycoprotein (VSG) gene expression with clonal expression of virulence in African trypanosomes was addressed. Two populations of clonally related trypanosomes, which differ dramatically in virulence for the infected host, but display the same apparent VSG surface coat phenotype, were characterized with respect to the VSG genes expressed as well as the chromosome telomeric expression sites (ES) utilized for VSG gene transcription. The VSG gene sequences expressed by clones LouTat 1 and LouTat 1A of *Trypanosoma brucei rhodesiense* were identical, and gene expression in both clones occurred precisely by the same gene conversion events (duplication and transposition), which generated an expression-linked copy (ELC) of the VSG gene. The ELC was present on the same genomic restriction fragments in both populations and resided in the telomere of a 330-kb chromosome; a single basic copy of the LouTat 1/1A VSG gene, present in all variants of the LouTat 1 serodeme, was located at an internal site of a 1.5-Mb chromosome. Restriction endonuclease mapping of the ES telomere revealed that the VSG ELC of clones LouTat 1 and 1A resides in the same site. Therefore, these findings provide evidence that the VSG gene ES and, potentially, any cotranscribed ES-associated genes do not play a role in the clonal regulation of virulence because trypanosome clones LouTat 1 and 1A, which differ markedly in their virulence properties, both express identical VSG genes from the same chromosome telomeric ES.

15286. **Jia, Y., Zhao, X., Zou, J. & Suo, X., 2010**. *Trypanosoma evansi*: identification and characterization of a variant surface glycoprotein lacking cysteine residues in its C-terminal domain. *Experimental Parasitology*. **In press, corrected proof.**

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African trypanosomes are flagellated unicellular parasites which proliferate extracellularly in the mammalian host blood-stream and tissue spaces. They evade the hosts' antibody-mediated lyses by sequentially changing their variant surface glycoprotein (VSG). VSG tightly coats the entire parasite body, serving as a physical barrier. In *Trypanosoma brucei* and the closely related species *Trypanosoma evansi* and *Trypanosoma equiperdum*, each VSG polypeptide can be divided into N- and C- terminal domains, based on cysteine distribution and sequence homology. N-terminal domain, the basis of antigenic variation, is hypervariable and contains all the exposed epitopes; the C-terminal domain is relatively conserved and a full set of 4 or 8 cysteines were generally observed. We cloned two genes from two distinct variants of *T. evansi*, utilizing RT-PCR with VSG-specific primers. One contained a VSG type A N-terminal domain followed a C-terminal domain lacking cysteine residues. To confirm that this gene is expressed as a functional VSG, the expression and localization of the corresponding gene product were characterized using western blotting and immunofluorescent staining of living trypanosomes. Expression analysis showed that this protein was highly expressed, variant-specific, and had a ubiquitous cellular surface localization. All these results indicated that it was expressed as a functional VSG. Our finding showed that cysteine residues in VSG C-terminal domain were not essential; the conserved C-terminal domain generally in *T. brucei* like VSGs would possibly evolve for regulating the VSG expression.

15287**. Koning, N., van Eijk, M., Pouwels, W., Brouwer, M. S., Voehringer, D., Huitinga, I., Hoek, R. M., Raes, G. & Hamann, J., 2010.** Expression of the inhibitory CD200 receptor is associated with alternative macrophage activation. *Journal of Innate Immunity,* **2** (2): 195-200.

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Classical macrophage activation is inhibited by the CD200 receptor (CD200R). Here, we show that CD200R expression was specifically induced on human *in vitro* polarized macrophages of the alternatively activated M2a subtype, generated by incubation with IL-4 or IL-13. In mice, peritoneal M2 macrophages, elicited during infection with the parasites *Taenia crassiceps* or *Trypanosoma brucei brucei*, expressed increased CD200R levels compared to those derived from uninfected mice. However, *in vitro* stimulation of mouse peritoneal macrophages and *T. crassiceps* infection in IL-4-/- and IL-4R-/- mice showed that, in contrast to humans, induction of CD200R in mice was not IL-4 or IL-13 dependent. Our data identify CD200R as a suitable marker for alternatively activated macrophages in humans and corroborate observations of distinct species- and/or site-specific mechanisms regulating macrophage polarization in mouse and man.

15288. **Lanca, A. S., de Sousa, K. P., Atouguia, J., Prazeres, D. M., Monteiro, G. A. & Silva, M. S., 2010.** *Trypanosoma brucei*: immunization with plasmid DNA encoding invariant surface glycoprotein gene is able to induce partial protection in experimental African trypanosomiasis. *Experimental Parasitology*. **e publication ahead of print June 18.**

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Trypanosoma brucei is the aetiological agent responsible for African trypanosomiasis, an infectious pathology which represents a serious problem of public health and economic losses in sub-Saharan Africa. As one of the foremost neglected illnesses, few resources have been available for the development of vaccines or new drugs, in spite of the current therapeutical drugs showing little efficiency and high toxicity. Hence, it is obviously important to widen effective therapeutics and preventive strategies against African trypanosomiasis. In this work, we use the DNA vaccine model to evaluate immunization effectiveness in mice challenged with *Trypanosoma brucei brucei*. We demonstrate that Balb/C mice immunized intramuscularly with a single dose of a DNA plasmid encoding a bloodstream stage specific invariant surface glycoprotein (ISG) are partially protected from a lethal dose of *Trypanosoma brucei brucei*. Interestingly, the surviving animals show high levels of IgG2a anti-trypanosomal antibodies, suggesting that the Th1 response profile seems important for the induced mechanisms of immune protection.

15289**. Lundkvist, G. B., Sellix, M. T., Nygard, M., Davis, E., Straume, M., Kristensson, K. & Block, G. D., 2010.** Clock gene expression during chronic inflammation induced by infection with *Trypanosoma brucei brucei* in rats. *Journal of Biological Rhythms,* **25** (2): 92-102.

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African sleeping sickness is characterized by alterations in rhythmic functions. It is not known if the disease affects the expression of clock genes, which are the molecular basis for rhythm generation. We used a chronic rat model of experimental sleeping sickness, caused by the extracellular parasite *Trypanosoma brucei brucei* (*Tb brucei*), to study the effects on clock gene expression. In tissue explants of pituitary glands from Period1-luciferase (Per1-luc) transgenic rats infected with *Tb brucei*, the period of Per1-luc expression was significantly shorter. In explants containing the suprachiasmatic nuclei (SCN), the Per1-luc rhythms were flat in 21 percent of the tissues. We also examined the relative expression of Per1, Clock, and Bmal1 mRNA in the SCN, pineal gland, and spleen from control and infected rats using qPCR. Both Clock and Bmal1 mRNA expression was reduced in the

pineal gland and spleen following *Tb brucei* infection. Infected rats were periodic both in core body temperature and in locomotor activity; however, early after infection, we observed a significant decline in the amplitude of the locomotor activity rhythm. In addition, both activity and body temperature rhythms exhibited decreased regularity and "robustness." In conclusion, although experimental trypanosome infection has previously been shown to cause functional disturbances in SCN neurons, only 21 percent of the SCN explants had disturbed Per1-luc rhythms. However, our data show that the infection overall alters molecular clock function in peripheral clocks including the pituitary gland, pineal gland, and spleen.

15290**. Magez, S., Caljon, G., Tran, T., Stijlemans, B. & Radwanska, M., 2010.** Current status of vaccination against African trypanosomiasis. *Parasitology.* **e Publication ahead of print May 11.**

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Anti-trypanosomiasis vaccination still remains the best theoretical option in the fight against a disease that is continuously hovering between its wildlife reservoir and its reservoir in man and livestock. While antigenic variation of the parasite surface coat has been considered the major obstacle in the development of a functional vaccine, recent research into the biology of B cells has indicated that the problems might go further than that. This paper reviews past and current attempts to design both anti-trypanosome vaccines, as well as vaccines directed towards the inhibition of infection-associated pathology.

15291. **Morrison, L. J., McLellan, S., Sweeney, L., Chan, C. N., MacLeod, A., Tait, A. & Turner, C. M., 2010**. Role for parasite genetic diversity in differential host responses to *Trypanosoma brucei* infection. *Infection & Immunity,* **78** (3): 1096-1108.

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The postgenomic era has revolutionized approaches to defining host-pathogen interactions and the investigation of the influence of genetic variation in either protagonist upon infection outcome. We analyzed the pathology induced by infection with two genetically distinct *Trypanosoma brucei* strains and found that pathogenesis is partly strain specific, involving distinct host mechanisms. Infections of BALB/c mice with one strain (927) resulted in more severe anaemia and greater erythropoietin production compared to infections with the second strain (247), which, contrastingly, produced greater splenomegaly and reticulocytosis. Plasma interleukin-10 (IL-10) and gamma interferon levels were significantly higher in strain 927-infected mice, whereas IL-12 was higher in strain 247-infected mice. To define mechanisms underlying these differences, expression microarray analysis of host genes in the spleen at day 10 postinfection was undertaken. Rank product

analysis (RPA) showed that 40 percent of the significantly differentially expressed genes were specific to infection with one or the other trypanosome strain. RPA and pathway analysis identified LXR/RXR signalling, IL-10 signalling, and alternative macrophage activation as the most significantly differentially activated host processes. These data suggest that innate immune response modulation is a key determinant in trypanosome infections, the pattern of which can vary, dependent upon the trypanosome strain. This strongly suggests that a parasite genetic component is responsible for causing disease in the host. Our understanding of trypanosome infections is largely based on studies involving single parasite strains, and our results suggest that an integrated host-parasite approach is required for future studies on trypanosome pathogenesis. Furthermore, it is necessary to incorporate parasite variation into both experimental systems and models of pathogenesis.

15292. **Otto, M. A., da Silva, A. S., Gressler, L. T., Farret, M. H., Tavares, K. C., Zanette, R. A., Miletti, L. C. & Monteiro, S. G., 2010.** Susceptibility of *Trypanosoma evansi* to human blood and plasma in infected mice. *Veterinary Parasitology,* **168** (1-2): 1-4.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Camobi, 9, Prédio 20, Sala 4232, CEP 97105900, Santa Maria, RS, Brazil; and Universidade do Estado de Santa Catarina, Lages, SC, Brazil. [sgmonteiro@uol.com.br].

Around 1900 Laveran and Mesnil discovered that African trypanosomes do not survive in the blood of some primates and humans. The nature of the trypanolytic factor present in these sera has been the focus of a long-standing debate between different groups. The aim of this study was to investigate the susceptibility of *T. evansi* isolates to therapy using human blood and plasma in experimentally infected mice. Forty-eight 2-month-old female mice (*Mus musculus*) were divided into six groups of eight animals per group (A, B, C, D, E and F). Plasma was obtained after blood collection in order to perform therapy. Animals from group A (positive control) were inoculated with *T. evansi* and treated with 0.2mL of saline solution. Animals from groups B and C were infected with the flagellate and received a curative treatment with 0.2mL of human blood (group B) and 0.2mL of human plasma (group C), 24h after infection. Animals from groups D and E received a prophylactic treatment with 0.2mL of human blood and 0.2mL of human plasma, respectively, 24h prior to the infection. Animals from group F (negative control) were not infected and received 0.2mL of saline solution. The four treatments (B, C, D and E) increased animals' longevity when compared to group A. Prepatency period was longer in groups D (15 days) and E (37.7 days) under prophylactic immunotherapy. Moreover, no parasites were found in most of the animals 60 days post-inoculation (PI). Besides the longer longevity, treatments were capable of curing 50 percent of mice of group B, 37.5 percent of group C, 37.5 percent of group D and 25 percent of the animals from group E.

15293. **Stijlemans, B., Vankrunkelsven, A., Brys, L., Raes, G., Magez, S. & De Baetselier, P., 2010.** Scrutinizing the mechanisms underlying the induction of anaemia of inflammation through GPI-mediated modulation of macrophage activation in a model of African trypanosomiasis. *Microbes & Infection,* **12** (5): 389-399.

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In animal trypanosomiasis the severity of infection is reflected by the degree of anaemia which resembles anaemia of inflammation, involving a skewed iron homeostasis leading to iron accumulation within the reticuloendothelial system. Myeloid cells (M cells) have been implicated in the induction and maintenance of this type of anaemia and modulation of M cells through the main trypanosome-derived glycosylphosphatidylinositol (GPI)-anchor could attenuate both anaemia and trypano-susceptibility in *Trypanosoma brucei*-infected mice. Herein the GPI-based treatment, allowing a straightforward comparison between trypanotolerance and susceptibility in *T. brucei*-infected C57Bl/6 mice, was further adopted to scrutinize mechanisms/pathways underlying trypanosome-elicited anaemia. Hereby, the following interlinkable observations were made in GPI-based treated (GBT) *T. brucei*-infected mice: (i) a reduced inflammatory cytokine production and increased IL-10 production associated with alleviation of anaemia and restoration of serum iron levels, (ii) a shift in increased liver expression of iron storage towards iron export genes, (iii) increased erythropoiesis in the bone marrow and extramedullar sites (spleen) probably reflecting a normalized iron homeostasis and availability. Collectively, our results demonstrate that reprogramming macrophages towards an anti-inflammatory state alleviates anaemia of inflammation by normalizing iron homeostasis and restoring erythropoiesis.

15294. **Stijlemans, B., Vankrunkelsven, A., Caljon, G., Bockstal, V., Guilliams, M., Bosschaerts, T., Beschin, A., Raes, G., Magez, S. & De Baetselier, P., 2010.** The central role of macrophages in trypanosomiasis-associated anaemia: rationale for therapeutical approaches. *Endocrine, Metabolic & Immune Disorders - Drug Targets,* **10** (1): 71-82.

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Bovine African trypanosomiasis causes severe economical problems on the African continent and one of the most prominent immunopathological parameters associated with this parasitic infection is anaemia. In this report we review the current knowledge of the mechanisms underlying trypanosomiasis-associated anaemia. In the first instance, the central role of macrophages and particularly their activation state in determining the outcome of the disease (i.e. trypanosusceptibility versus trypanotolerance) will be discussed. In essence, while persistence of classically activated macrophages (M1) contributes to anaemia development, switching towards alternatively activated macrophages (M2) alleviates pathology including anaemia. Secondly, while parasite-derived glycolipids such as the glycosylphosphatidylinositol (GPI) induce M1, host-derived IL-10 blocks M1-mediated inflammation, promotes M2 development and prevents anaemia development. In this context, strategies aimed at inducing the M1 to M2 switch, such as GPI-based treatment, adenoviral delivery of IL-10 and induction of IL-10 producing regulatory T cells will be discussed. Finally, the crucial role of iron homeostasis in trypanosomiasis-associated anaemia development will be documented to stress the analogy with anaemia of chronic disease (ACD), hereby providing new insight that might contribute to the treatment of ACD.

(c) CHEMOTHERAPEUTICS

[See also **33**: 15294,].

15295. **Bakunov, S. A., Bakunova, S. M., Wenzler, T., Ghebru, M., Werbovetz, K. A., Brun, R. & Tidwell, R. R., 2010.** Synthesis and antiprotozoal activity of cationic 1,4-diphenyl-1H-1,2,3-triazoles. *Journal of Medicinal Chemistry,* **53** (1): 254-272.

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Novel dicationic triazoles 1-60 were synthesized by the Pinner method from the corresponding dinitriles, prepared via the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). The type and the placement of cationic moieties as well as the nature of aromatic substituents influenced *in vitro* antiprotozoal activities of compounds 1-60 against *Trypanosoma brucei rhodesiense, Plasmodium falciparum*, and *Leishmania donovani* and their cytotoxicity for mammalian cells. Eight congeners displayed antitrypanosomal IC_{50} values below 10 nM. Thirty-nine dications were more potent against *P. falciparum* than pentamidine (IC_{50} = 58 nM), and eight analogues were more active than artemisinin (IC_{50} = 6 nM). Diimidazoline 60 exhibited antiplasmodial IC_{50} value of 0.6 nM. Seven congeners administered at 4 x 5 mg/kg by the intraperitoneal route cured at least three out of four animals in the acute mouse model of African trypanosomiasis. At 4 x 1 mg/kg, diamidine 46 displayed better antitrypanosomal efficacy than melarsoprol, curing all infected mice.

15296. **Bawm, S., Tiwananthagorn, S., Lin, K. S., Hirota, J., Irie, T., Htun, L. L., Maw, N. N., Myaing, T. T., Phay, N., Miyazaki, S., Sakurai, T., Oku, Y., Matsuura, H. & Katakura, K., 2010.** Evaluation of Myanmar medicinal plant extracts for antitrypanosomal and cytotoxic activities. *Journal of Veterinary Medical Science,* **72** (4): 525-528.

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Current chemotherapeutic options for African trypanosomiasis in humans and livestock are very limited. In the present study, a total of 71 medicinal plant specimens from 60 plant species collected in Myanmar were screened for antitrypanosomal activity against trypomastigotes of *Trypanosoma evansi* and cytotoxicity against MRC-5 cells *in vitro*. The methanol extract of dried rootbark of *Vitis repens* showed the highest antitrypanosomal activity with an IC_{50} value of 8.6 +/- 1.5 µg/mL and the highest selectivity index of 24.4. The extracts of *Brucea javanica, Vitex arborea, Eucalyptus globulus* and *Jatropha podagrica* had also remarkable activity with IC_{50} values and selectivity indices in the range of 27.2-52.6 µg/mL and 11.4-15.1 respectively.

15297. **Berg, M., Kohl, L., Van der Veken, P., Joossens, J., Al-Salabi, M. I., Castagna, V., Giannese, F., Cos, P., Versees, W., Steyaert, J., Grellier, P., Haemers, A., Degano, M., Maes, L., de Koning, H. P. & Augustyns, K., 2010.** Evaluation of

nucleoside hydrolase inhibitors for treatment of African trypanosomiasis. *Antimicrobial Agents & Chemotherapy,* **54** (5): 1900-1908.

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In this paper, we present the biochemical and biological evaluation of N-arylmethyl-substituted iminoribitol derivatives as potential chemotherapeutic agents against trypanosomiasis. Previously, a library of 52 compounds was designed and synthesized as potent and selective inhibitors of *Trypanosoma vivax* inosine-adenosine-guanosine nucleoside hydrolase (IAG-NH). However, when the compounds were tested against bloodstream-form *Trypanosoma brucei brucei*, only one inhibitor, N-(9-deaza-adenin-9-yl)methyl-1,4-dideoxy-1,4-imino-d-ribitol (UAMC-00363), displayed significant activity (mean 50 percent inhibitory concentration [IC_{50}] +/- standard error, 0.49 +/- 0.31 µM). Validation in an *in vivo* model of African trypanosomiasis showed promising results for this compound. Several experiments were performed to investigate why only UAMC-00363 showed antiparasitic activity. First, the compound library was screened against *T. b. brucei* IAG-NH and inosine-guanosine nucleoside hydrolase (IG-NH) to confirm the previously demonstrated inhibitory effects of the compounds on *T. vivax* IAG-NH. Second, to verify the uptake of these compounds by *T. b. brucei*, their affinities for the nucleoside P1 and nucleoside/nucleobase P2 transporters of *T. b. brucei* were tested. Only UAMC-00363 displayed significant affinity for the P2 transporter. It was also shown that UAMC-00363 is concentrated in the cell via at least one additional transporter, since P2 knockout mutants of *T. b. brucei* displayed no resistance to the compound. Consequently, no cross-resistance to the diamidine or the melaminophenyl arsenical classes of trypanocides is expected. Third, three enzymes of the purine salvage pathway of procyclic *T. b. brucei* (IAG-NH, IG-NH, and methylthioadenosine phosphorylase [MTAP]) were investigated using RNA interference. The findings from all these studies showed that it is probably not sufficient to target only the nucleoside hydrolase activity to block the purine salvage pathway of *T. b. brucei* and that, therefore, it is possible that UAMC-00363 acts on an additional target.

15298**. Berg, M., Van der Veken, P., Goeminne, A., Haemers, A. & Augustyns, K., 2010.** Inhibitors of the purine salvage pathway: a valuable approach for antiprotozoal chemotherapy? *Current Medicinal Chemistry,* **17** (23): 2456-2481.

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For many years, the purine salvage pathway of parasitic protozoa has been regarded as an attractive chemotherapeutic target. Parasitic protozoa lack *de novo* synthesis and rely entirely on the purine salvage pathway to meet their purine demands. Because of the great phylogenetic difference between parasite and host, there are often sufficient distinctions that can be exploited to design specific inhibitors for the parasitic enzymes. As a result, this pathway has been thoroughly investigated over the last twenty years. It is only quite recently that the genome studies of *Trypanosoma, Leishmania* and *Plasmodium* have been published. Based on these genomic data however, the existence of by-pass mechanisms by other enzymes and transporter systems could be suggested. Taking into account such a proposition,

the question might arise as to whether inhibition of a single salvage enzyme will be able or not to cause parasite death or growth arrest. In this paper, the key enzymes in the purine salvage pathways of relevant pathogenic species from the genera *Trypanosoma, Leishmania* and *Plasmodium* are reviewed. Their potential as drug targets is critically evaluated and where possible, correlated to literature data on antiparasitic activity of their inhibitors. While many studies over the past ten years have yielded contradictory results, this review attempts to clarify these findings by discussing the latest elements of progress in the field. Additionally, as part of a broader discussion on substrate analogue types of inhibitors, special attention is paid to iminoribitol derivatives, serving as transition state analogues of nucleoside-processing enzymes and comprising the most potent inhibitors reported for purine salvage enzymes. More specifically, the development of three generations of immucillins and a newer series of N-(arylmethyl-) substituted iminoribitol derivatives will be discussed. Finally, this review also covers subversive substrates of salvage enzymes: compounds that are transformed by enzymatic activity into cytotoxic agents. Although not by directly intervening in the process of purine recovery, the subversive substrate approach might deliver antiprotozoal compounds that rely on salvage enzymes for their activity.

15299. **Branowska, D., Farahat, A. A., Kumar, A., Wenzler, T., Brun, R., Liu, Y., Wilson, W. D. & Boykin, D. W., 2010.** Synthesis and antiprotozoal activity of 2,5-bis[amidinoaryl]thiazoles. *Bioorganic & Medicinal Chemistry,* **18** (10): 3551-3558.

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Seven novel diamidino 2,5-bis(aryl)thiazoles (5a-g) were synthesized and evaluated against *Trypanosoma brucei rhodensiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*). The diamidines were obtained directly from the corresponding bis-nitriles (4a-g) by the action of lithium bis(trimethylsilyl)amide. The bis-nitriles 4a-f were synthesized in four steps starting with the Stille coupling of 2-tributyltinthiazole with the appropriate cyanoaryl halide. The bis-nitrile 5g was obtained by the palladium facilitated coupling of the mixed tin-silyl reagent 2-trimethylsilyl-5-trimethyltinthiazole with 2-bromo-5-cyanopyridine. The amidoxime potential prodrugs 6a-e, 6g were obtained by the reaction of hydroxylamine with the bis-nitriles. O-methylation of the amidoximes gave the corresponding N-methoxyamidines 7a-c, 7e, 7g. The diamidines showed strong DNA binding affinity as reflected by DeltaT_m measurements. Four of the diamidines 5a, 5b, 5d and 5e were highly active *in vitro* against *P. f.* giving IC_{50} values between 1.1 and 2.5nM. The same four diamidines showed IC_{50} values between 4 and 6nM against *T. b. r.* The selectivity indices ranged from 233 to 9175. One diamidine 5a produced one of four cures at an ip dose of 4x5mg/kg in the STIB900 mouse model for acute African trypanosomiasis. The amidoxime and N-methoxyamidine of 5a were the only produgs to provide cures (1/4 cures) in the same mouse model on oral dosage at 4x25mg/kg.

15300. **Caceres, A. J., Michels, P. A. & Hannaert, V., 2010.** Genetic validation of aldolase and glyceraldehyde-3-phosphate dehydrogenase as drug targets in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **169** (1): 50-54.

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Aldolase (ALD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *Trypanosoma brucei* are considered to be promising targets for chemotherapeutic treatment of African sleeping sickness, because glycolysis is the single source of ATP for the parasite when living in the human bloodstream. Moreover, these enzymes appeared to possess distinct kinetic and structural properties that have already been exploited for the discovery of effective and selective inhibitors with trypanocidal activity. Here we present an experimental, quantitative assessment of the importance of these enzymes for the glycolytic pathway. This was achieved by decreasing the concentrations of ALD and GAPDH by RNA interference. The effects of these knockdowns on parasite growth, levels of various enzymes and transcripts, enzyme activities and glucose consumption were studied. A partial depletion of ALD and GAPDH was already sufficient to rapidly kill the trypanosomes. An effect was also observed on the activity of some other glycolytic enzymes.

15301. **Chavda, S., Babu, B., Yanow, S. K., Jardim, A., Spithill, T. W., Kiakos, K., Kluza, J., Hartley, J. A. & Lee, M., 2010.** A novel achiral seco-cyclopropylpyrido[e]indolone (CPyI) analogue of CC-1065 and the duocarmycins: synthesis, DNA interactions, *in vivo* anticancer and anti-parasitic evaluation. *Bioorganic & Medicinal Chemistry,* **18** (14): 5016-5024

Division of Natural Sciences and Department of Chemistry, Hope College, 35 East 12th Street, Holland, MI 49423, USA. [lee@hope.edu].

15302. **Chen, C. K., Leung, S. S., Guilbert, C., Jacobson, M. P., McKerrow, J. H. & Podust, L. M., 2010.** Structural characterization of CYP51 from *Trypanosoma cruzi* and *Trypanosoma brucei* bound to the antifungal drugs posaconazole and fluconazole. *PLoS Neglected Tropical Diseases,* **4** (4): e651.

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15303**. Cross, G. A., 2010.** Drug discovery: fat-free proteins kill parasites. *Nature,* **464** (7289): 689-690.

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The addition of a fatty acid to certain proteins is vital for the survival of protozoa that cause sleeping sickness and of their mammalian hosts. Compounds that target this process in the protozoa are now reported.

15304. **Davis, R. A., Demirkiran, O., Sykes, M. L., Avery, V. M., Suraweera, L., Fechner, G. A. & Quinn, R. J., 2010**. 7',8'-Dihydroobolactone, a trypanocidal alpha-pyrone from the rainforest tree *Cryptocarya obovata*. *Bioorganic & Medicinal Chemistry Letters,* **20** (14): 4057-4059.

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Mass-directed isolation of the CH_2Cl_2/MeOH extract from the leaves of *Cryptocarya obovata* resulted in the purification of a new trypanocidal alpha-pyrone, 7',8'-dihydroobolactone (1). The chemical structure of 1 was determined by 1D/2D NMR, MS and CD data analysis. 7',8'-Dihydroobolactone was shown to inhibit *Trypanosoma brucei brucei* with an IC_{50} of 2.8 µM.

15305. **Debierre-Grockiego, F., 2010**. Glycolipids are potential targets for protozoan parasite diseases. *Trends in Parasitology,* **26** *(*8): 404-411.

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Induction of sterilizing immunity by vaccination is extremely difficult because of the evasion mechanisms developed by parasites, and identification of new targets for therapy is therefore important. Glycosylphosphatidylinositols (GPIs) of parasites are glycolipids that participate in pathogenicity of parasitic diseases. Studies of *Plasmodium falciparum* and *Trypanosoma brucei* indicate that GPIs are good candidates for developing vaccines against malaria and sleeping sickness, respectively. By contrast, fatty acids isolated from *P. falciparum* and *Toxoplasma gondii* can inhibit the production of inflammatory cytokines induced by the GPIs in macrophages. GPIs are considered to be toxins that, if present in large amounts, induce irreversible damages to the host, and treatment with fatty acids could reduce this effect.

15306. **Durrant, J. D., Urbaniak, M. D., Ferguson, M. A. & McCammon, J. A., 2010.** Computer-aided identification of *Trypanosoma brucei* uridine diphosphate galactose 4'-epimerase inhibitors: toward the development of novel therapies for African sleeping sickness. *Journal of Medicinal Chemistry,* **53** (13): 5025-5032.

Biomedical Sciences Program, University of California San Diego, 9500 Gilman Drive, Mail Code 0365, La Jolla, California 92093-0365, USA. [jdurrant@ucsd.edu].

Trypanosoma brucei, the causative agent of human African trypanosomiasis, affects tens of thousands of sub-Saharan Africans. As current therapeutics are inadequate due to toxic side effects, drug resistance, and limited effectiveness, novel therapies are urgently needed. UDP-galactose 4'-epimerase (TbGalE), an enzyme of the Leloir pathway of galactose metabolism, is one promising *T. brucei* drug target. Here we use the relaxed complex scheme, an advanced computer-docking methodology that accounts for full protein flexibility, to identify inhibitors of TbGalE. An initial hit rate of 62 percent was obtained at 100 µM, ultimately leading to the identification of 14 low-micromolar inhibitors. Thirteen of these inhibitors belong to a distinct series with a conserved binding motif that may prove useful in future drug design and optimization.

15307. **Fernandez, L. S., Sykes, M. L., Andrews, K. T. & Avery, V. M., 2010.** Antiparasitic activity of alkaloids from plant species of Papua New Guinea and Australia. *International Journal of Antimicrobial Agents,* **36** (3): 275-279.

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Australia; and Griffith Medical Research College, A Joint Program of Griffith University and the Queensland Institute of Medical Research, QIMR, Herston, QLD 4006, Australia. [v.avery@griffith.edu.au].

New drugs are needed to help overcome the increasing problem of drug resistance in parasites that cause diseases such as malaria and trypanosomiasis. In this study, alkaloid compounds isolated from extracts of the plants *Flindersia amboinensis, Stephania zippeliana* and *Voacanga papuana* from Papua New Guinea and *Flindersia acuminata* from Australia were examined for their antiparasitic activity against *Plasmodium falciparum* strains and *Trypanosoma brucei brucei* as well as their cytotoxicity against the mammalian cell lines HEK 293 and HeLa. The most active compound, dimethylisoborreverine (DMIB), showed sub micromolar activity, with 50 percent inhibitory concentration (IC_{50}) values between 20nM and 810nM both against drug-sensitive and drug-resistant *P. falciparum* strains, along with moderate selectivity against *T. b. brucei* and mammalian cells. Stage specificity studies revealed that *P. falciparum* trophozoite-stage parasites were more susceptible to DMIB than ring- or schizont-stage parasites. DMIB-treated trophozoites showed changes in food vacuole morphology, with an apparent reduction in haemozoin formation that does not appear to be inhibited via the direct binding of haem. These findings suggest a potential for indole alkaloids from *Flindersia* spp. as new antiparasitic agents.

15308. **Fotie, J., Kaiser, M., Delfin, D. A., Manley, J., Reid, C. S., Paris, J. M., Wenzler, T., Maes, L., Mahasenan, K. V., Li, C. & Werbovetz, K. A., 2010.** Antitrypanosomal activity of 1,2-dihydroquinolin-6-ols and their ester derivatives. *Journal of Medicinal Chemistry,* **53** (3): 966-982.

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The current chemotherapy for second stage human African trypanosomiasis is unsatisfactory. A synthetic optimization study based on the lead antitrypanosomal compound 1,2-dihydro-2,2,4-trimethylquinolin-6-yl 3,5-dimethoxybenzoate (TDR20364, 1a) was undertaken in an attempt to discover new trypanocides with potent *in vivo* activity. While 6-ether derivatives were less active than the lead compound, several N1-substituted derivatives displayed nanomolar IC_{50} values against *T. b. rhodesiense* STIB900 *in vitro*, with selectivity indexes up to >18 000. 1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (10a) displayed an IC_{50} value of 0.014 µM against these parasites and a selectivity index of 1 700. Intraperitoneal administration of 10a at 50 mg/kg/day for 4 days caused a promising prolongation of lifespan in *T. b. brucei* STIB795-infected mice (>14 days vs 7.75 days for untreated controls). Reactive oxygen species were produced when *T. b. brucei* were exposed to 10a *in vitro*, implicating oxidative stress in the trypanocidal mode of action of these 1,2-dihydroquinoline derivatives.

15309. **Frearson, J. A., Brand, S., McElroy, S. P., Cleghorn, L. A., Smid, O., Stojanovski, L., Price, H. P., Guther, M. L., Torrie, L. S., Robinson, D. A., Hallyburton, I., Mpamhanga, C. P., Brannigan, J. A., Wilkinson, A. J., Hodgkinson, M., Hui, R., Qiu, W., Raimi, O. G., van Aalten, D. M., Brenk, R., Gilbert, I. H., Read, K. D., Fairlamb, A. H., Ferguson, M. A., Smith, D. F. & Wyatt, P. G., 2010**. N-myristoyltransferase inhibitors as new leads to treat sleeping sickness. *Nature*, **464** (7289): 728-732.

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African sleeping sickness or human African trypanosomiasis, caused by *Trypanosoma brucei* spp., is responsible for approximately 30 000 deaths each year. Available treatments for this disease are poor, with unacceptable efficacy and safety profiles, particularly in the late stage of the disease when the parasite has infected the central nervous system. Here we report the validation of a molecular target and the discovery of associated lead compounds with the potential to address this lack of suitable treatments. Inhibition of this target-*T. brucei* N-myristoyltransferase-leads to rapid killing of trypanosomes both *in vitro* and *in vivo* and cures trypanosomiasis in mice. These high-affinity inhibitors bind into the peptide substrate pocket of the enzyme and inhibit protein N-myristoylation in trypanosomes. The compounds identified have promising pharmaceutical properties and represent an opportunity to develop oral drugs to treat this devastating disease. Our studies validate *T. brucei* N-myristoyltransferase as a promising therapeutic target for human African trypanosomiasis.

15310. **Goldsmith, R. B., Gray, D. R., Yan, Z., Generaux, C. N., Tidwell, R. R. & Reisner, H. M., 2010.** Application of monoclonal antibodies to measure metabolism of an anti-trypanosomal compound *in vitro* and *in vivo*. *Journal of Clinical Laboratory Analysis,* **24** (3): 187-194.

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Human African trypanosomiasis (HAT), also called African sleeping sickness, is a neglected tropical parasitic disease indigenous to sub-Saharan Africa. Diamidine compounds, including pentamidine and CPD-0801, are potent anti-trypanosomal molecules. The latter is a potential drug in the development at the UNC based Consortium for Parasitic Drug Development. An orally bioavailable prodrug of CPD-0801, DB868, is metabolized primarily in the liver to the active form. A monoclonal antibody developed against a pentamidine derivative has shown significant reactivity with CPD-0801 (EC_{50} 65.1 nM), but not with the prodrug (EC_{50} > 18 000 nM). An inhibitory enzyme-linked immunosorbent assay (IELISA) has been used to quantitatively monitor prodrug metabolism by detecting the production of the active compound over time in a sandwich culture rat hepatocyte system and in rats. These results were compared with the results of the standard LC/MS/MS assay. Spearman coefficients of 0.96 and 0.933 (*in vitro* and *in vivo*, respectively) indicate a high correlation between these two measurement methods. This novel IELISA provides a facile, inexpensive, and accurate method for drug detection that may aid in elucidating the mechanisms of action and toxicity of existing and future diamidine compounds.

15311. **Hagos, A., Goddeeris, B. M., Yilkal, K., Alemu, T., Fikru, R., Yacob, H. T., Feseha, G. & Claes, F., 2010.** Efficacy of Cymelarsan® and Diminasan® against *Trypanosoma equiperdum* infections in mice and horses. *Veterinary Parasitology,* 171 (3-4): 200-206.

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Trypanocidal sensitivity studies were conducted to assess the efficacy of diminazene diaceturate (Diminasan®) and bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®) against *Trypanosoma equiperdum* (isolated from two mares with chronic cases of dourine) 713/943 and 834/940 Dodola strains in experimentally infected mice and horses. Diminasan® at doses from 3.5mg/kg to 28mg/kg and Cymelarsan® at doses of 0.25mg/kg and 0.5mg/kg body weight failed to cure any of the mice, indicating a clear dose dependent relationship in the mean time of relapse observed in mice. Indeed, mice treated with lower doses relapsed after a shorter time than mice treated with higher doses. However, mice treated with Cymelarsan® at doses of 1.0mg/kg and 2.0mg/kg body weight were cured and no parasitaemia was observed for 60 days. The efficacy of Cymelarsan® was also tested in horses. Two groups of horses containing two animals each were infected with *T. equiperdum* 834/940 Dodola strain and treated with Cymelarsan® at a dose rate of 0.25mg/kg and 0.5mg/kg, respectively. Cymelarsan® at 0.25mg/kg and 0.5mg/kg body weight cleared parasitaemia within 24h post treatment and none of the animals were found to show relapse throughout the 320 days of observation. The sensitivity of the particular trypanosome strain to Cymelarsan® was also supported by the relative improvement in the mean PCV levels of horses following treatment. A statistically significant difference ($P < 0.01$) in the mean PCV levels of horses treated with Cymelarsan® was observed between day 20 at peak parasitaemia and days 40 as well as 60 of observation. The mean PCV levels of horses in the control group progressively decreased within the first 60 days of post infection. Two of the horses in the control group developed chronic form of dourine manifested by genital as well as nervous signs with progressive loss of body condition within 320 days post infection. The efficacy of Cymelarsan® against the chronic form of dourine was confirmed after treatment of one of the control horses with Cymelarsan® at a dose rate of 0.25mg/kg body weight at day 282 post infection. It was noted that the treated horse showed an improved overall body condition and clinical signs such as incoordination of hind legs, weakness and ventral oedema disappeared within 10 days of treatment. Thus, Cymelarsan® was found to be quite effective in curing horses with acute as well as the chronic form of dourine. The results obtained from the present study will be important for designing effective control measures against dourine.

15312. **Hwang, J. Y., Smithson, D., Connelly, M., Maier, J., Zhu, F. & Guy, K. R., 2010.** Discovery of halo-nitrobenzamides with potential application against human African trypanosomiasis. *Bioorganic & Medicinal Chemistry Letters,* **20** (1): 149-152.

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A series of halo-nitrobenzamide were synthesized and evaluated for their ability to block proliferation of *Trypanosoma brucei brucei*. A number of these compounds had significant activity against the parasite, particularly 2-chloro-N-(4-chlorophenyl)-5-nitrobenzamide 17 which exhibited low micromolar inhibitory potency against *T. brucei* and selectivity towards both malaria and mammalian cells.

15313. **Jones, D. C., Ariza, A., Chow, W. H., Oza, S. L. & Fairlamb, A. H., 2010.** Comparative structural, kinetic and inhibitor studies of *Trypanosoma brucei* trypanothione reductase with *T. cruzi*. *Molecular & Biochemical Parasitology,* **169** (1): 12-19.

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As part of a drug discovery programme to discover new treatments for human African trypanosomiasis, recombinant trypanothione reductase from *Trypanosoma brucei* has been expressed, purified and characterized. The crystal structure was solved by molecular replacement to a resolution of 2.3A and found to be nearly identical to the *T. cruzi* enzyme (root mean square deviation 0.6A over 482 Calpha atoms). Kinetically, the K_m for trypanothione disulphide for the *T. brucei* enzyme was 4.4-fold lower than for *T. cruzi* measured by either direct (NADPH oxidation) or DTNB-coupled assay. The K(m) for NADPH for the *T. brucei* enzyme was found to be 0.77µM using an NADPH-regenerating system coupled to reduction of DTNB. Both enzymes were assayed for inhibition at their respective $S=K_m$ values for trypanothione disulphide using a range of chemotypes, including CNS-active drugs such as clomipramine, trifluoperazine, thioridazine and citalopram. The relative IC_{50} values for the two enzymes were found to vary by no more than 3-fold. Thus trypanothione reductases from these species are highly similar in all aspects, indicating that they may be used interchangeably for structure-based inhibitor design and high-throughput screening.

15314. **Kerr, I. D., Wu, P., Marion-Tsukamaki, R., Mackey, Z. B. & Brinen, L. S., 2010.** Crystal structures of TbCatB and rhodesain, potential chemotherapeutic targets and major cysteine proteases of *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases,* **4** (6): e701.

Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, California, USA.. [brinen@cmp.ucsf.edu].

15315. **Kido, Y., Sakamoto, K., Nakamura, K., Harada, M., Suzuki, T., Yabu, Y., Saimoto, H., Yamakura, F., Ohmori, D., Moore, A., Harada, S. & Kita, K., 2010**. Purification and kinetic characterization of recombinant alternative oxidase from *Trypanosoma brucei brucei*. *Biochimica et Biophysica Acta,* **1797** (4): 443-450.

Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan. [kitak@m.u-tokyo.ac.jp].

15316. **Kido, Y., Shiba, T., Inaoka, D. K., Sakamoto, K., Nara, T., Aoki, T., Honma, T., Tanaka, A., Inoue, M., Matsuoka, S., Moore, A., Harada, S. & Kita, K., 2010.** Crystallization and preliminary crystallographic analysis of cyanide-insensitive alternative oxidase from *Trypanosoma brucei brucei*. *Acta Crystallographica Section F Structural Biology & Crystalization Communications,* **66** (Pt 3): 275-278.

Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan.

15317. **Klee, N., Wong, P. E., Baragana, B., Mazouni, F. E., Phillips, M. A., Barrett, M. P. & Gilbert, I. H., 2010.** Selective delivery of 2-hydroxy APA to *Trypanosoma brucei* using the melamine motif. *Bioorganic and Medicinal Chemistry Letters,* **20** (15): 4364-4366.

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Trypanosoma brucei, the parasite that causes human African trypanosomiasis, is auxotrophic for purines and has specialist nucleoside transporters to import these metabolites. In particular, the P2 aminopurine transporter can also selectively accumulate melamine derivatives. In this Letter, we report the coupling of the melamine moiety to 2-hydroxy APA, a potent ornithine decarboxylase inhibitor, with the aim of selectively delivering this compound to the parasite. The best compound described here shows an increased *in vitro* trypanocidal activity compared with the parent.

15318. **Lepesheva, G. I., Park, H. W., Hargrove, T. Y., Vanhollebeke, B., Wawrzak, Z., Harp, J. M., Sundaramoorthy, M., Nes, W. D., Pays, E., Chaudhuri, M., Villalta, F. & Waterman, M. R., 2010.** Crystal structures of *Trypanosoma brucei* sterol 14alpha-demethylase and implications for selective treatment of human infections. *Journal of Biological Chemistry,* **285** (3): 1773-1780.

Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37232, USA. [galina.i.lepesheva@vanderbilt.edu].

15319. **Mott, B. T., Ferreira, R. S., Simeonov, A., Jadhav, A., Ang, K. K., Leister, W., Shen, M., Silveira, J. T., Doyle, P. S., Arkin, M. R., McKerrow, J. H., Inglese, J., Austin, C. P., Thomas, C. J., Shoichet, B. K. & Maloney, D. J., 2010.** Identification and optimization of inhibitors of trypanosomal cysteine proteases: cruzain, rhodesain, and TbCatB. *Journal of Medicinal Chemistry,* **53** (1): 52-60.

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Trypanosoma cruzi and *Trypanosoma brucei* are parasites that cause Chagas disease and African sleeping sickness, respectively. Both parasites rely on essential cysteine proteases for survival: cruzain for *T. cruzi* and TbCatB/rhodesain for *T. brucei*. A recent quantitative high-throughput screen of cruzain identified triazine nitriles, which are known inhibitors of other cysteine proteases, as reversible inhibitors of the enzyme. Structural modifications detailed herein, including core scaffold modification from triazine to purine, improved the *in vitro* potency against both cruzain and rhodesain by 350-fold, while also gaining activity against *T. brucei* parasites. Selected compounds were screened against a panel of human cysteine and serine proteases to determine selectivity, and a cocrystal was obtained of our most potent analogue bound to cruzain.

15320. **Ngantchou, I., Nyasse, B., Denier, C., Blonski, C., Hannaert, V. & Schneider, B., 2010.** Antitrypanosomal alkaloids from *Polyalthia suaveolens* (Annonaceae): their effects on three selected glycolytic enzymes of *Trypanosoma brucei. Bioorganic & Medicinal Chemistry Letters,* **20** (12): 3495-3498.

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In continuation of our study on medicinal plants of Cameroon, stem barks of *Polyalthia suaveolens* were phytochemically studied. This investigation yielded a new indolosesquiterpene alkaloid, named polysin (1) and four hitherto known alkaloids (2-5). Polysin (1) appeared as a competitive reversible inhibitor (K_i = 10 µM) of phosphofructo kinase (PFK) of *Trypanosoma brucei* with respect to fructose-6-phosphate (K_i/K_M = 0.05) and could be used in the design of new trypanocidal drugs. The other isolated compounds (2-5) also exhibited interesting inhibitory effects on selected glycolytic enzymes (PFK, glyceraldehyde-3-phosphate dehydrogenase and aldolase).

15321. **Nour, A. M., Khalid, S. A., Kaiser, M., Brun, R., Abdalla, W. E. & Schmidt, T. J., 2010.** The antiprotozoal activity of methylated flavonoids from *Ageratum conyzoides L. Journal of Ethnopharmacology,* **129** (1): 127-130.

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The dichloromethane extract prepared from aerial parts of *Ageratum conyzoides* L. (Asteraceae), a plant commonly used in folk medicine for a number of illnesses including sleeping sickness, was recently found to exhibit a prominent activity (IC_{50}=0.78 µg/mL) against bloodstream forms of *Trypanosoma brucei rhodesiense*, the aetiologic agent of human African trypanosomiasis (East African sleeping sickness). This extract also exhibited noticeable activities against *Leishmania donovani* (Kala-Azar, IC_{50} = 3.4 µg/mL) as well as *Plasmodium falciparum* (IC_{50} = 8.0 µg/mL). In the current study, we sought potentially active

constituents of *Ageratum conyzoides*. Extracts prepared with solvents of different polarity were tested for activity against the above mentioned parasites as well as against *Trypanosoma cruzi* (Chagas disease) and for cytotoxicity using established protocols. The dicholoromethane extract showed the highest level of activity and was chosen for phytochemical studies aimed at the isolation of potential active constituents. Five highly methoxylated flavonoids along with the chromene derivative encecalol methyl ether were isolated. All isolated compounds were previously reported from *Ageratum conyzoides*. While the chromene turned out to be inactive against the tested parasites, the flavonoids showed activity against the protozoan pathogens, some in the lower µM range. However, none of these isolated compounds was as active as the crude extract. This is the first report on antiprotozoal activity of this plant species and some of its constituents. The chemical principle accounting for the high activity of the crude extract, however, remains to be identified.

15322**. Oldfield, E., 2010.** Targeting isoprenoid biosynthesis for drug discovery: bench to bedside. *Accounts of Chemical Research***. e publication June 18**.

Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.

15323. **Otoguro, K., Ishiyama, A., Iwatsuki, M., Namatame, M., Nishihara-Tukashima, A., Nakashima, T., Shibahara, S., Kondo, S., Yamada, H. & Omura, S., 2010**. *In vitro* and *in vivo* anti-*Trypanosoma brucei* activities of phenazinomycin and related compounds. *Journal of Antibiotics (Tokyo)*. **Advance online publication 30 June**.

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During the course of our screening programme to discover new antitrypanosomal compounds, we have evaluated isolates from soil microorganisms as well as compounds from the antibiotic libraries of the Kitasato Institute for Life Sciences and Bioscience Associates. We have previously reported on various microbial metabolites exhibiting potent anti-*Trypanosoma brucei* properties, which are defined as antitrypanosomal properties.

15324. **Regalado, E. L., Tasdemir, D., Kaiser, M., Cachet, N., Amade, P. & Thomas, O. P., 2010**. Antiprotozoal steroidal saponins from the marine sponge *Pandaros acanthifolium*. *Journal of Natural Products*. **Web publication July 8.**

Department of Chemistry, Center of Marine Bioproducts (CEBIMAR), Loma y 37 Alturas del Vedado, C.P. 10400 Havana, Cuba; Centre for Pharmacognosy and Phytotherapy, Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK; Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, 4002, Basel, Switzerland; and Laboratoire de Chimie des Molécules Bioactives et des Arômes, UMR 6001 CNRS, Institut de Chimie de Nice, Faculté des Sciences, University of Nice-Sophia Antipolis, Parc Valrose, 06108 Nice Cedex 2, France. [olivier.thomas@unice.fr].

The chemical composition of the Caribbean sponge *Pandaros acanthifolium* was reinvestigated and led to the isolation of 12 new steroidal glycosides, namely, pandarosides E-J (1-6) and their methyl esters (7-12). Their structures were determined on the basis of extensive spectroscopic analyses, including two-dimensional NMR and HRESIMS data. Like the previously isolated pandarosides A-D (13-16), the new compounds 1-12 share an unusual oxidized D-ring and a cis C/D ring junction. The absolute configurations of the aglycones were assigned by interpretation of CD spectra, whereas the absolute configurations of the monosaccharide units were determined by chiral GC analyses of the acid methanolysates. The majority of the metabolites showed *in vitro* activity against three or four parasitic protozoa. Particularly active were the compounds 3 (pandaroside G) and its methyl ester (9), which potently inhibited the growth of *Trypanosoma brucei rhodesiense* (IC_{50} values 0.78 and 0.038 μM, respectively) and *Leishmania donovani* (IC_{50} values of 1.3 and 0.051 μM, respectively).

15325**. Rodrigues, C., Batista, A. A., Ellena, J., Castellano, E. E., Benitez, D., Cerecetto, H., Gonzalez, M., Teixeira, L. R. & Beraldo, H., 2010.** Coordination of nitro-thiosemicarbazones to ruthenium (II) as a strategy for anti-trypanosomal activity improvement. *European Journal of Medicinal Chemistry,* **45** (7): 2847-2853.

Departamento de Quimica, Universidade Federal de Sao Carlos, 13565-905 Sao Carlos (SP), Brazil. [lregina@qui.ufmg.br].

15326. **Rodriguez-Soca, Y., Munteanu, C. R., Dorado, J., Pazos, A., Prado-Prado, F. J. & Gonzalez-Diaz, H., 2010.** Trypano-PPI: a web server for prediction of unique targets in the trypanosome proteome by using electrostatic parameters of protein-protein interactions. *Journal of Proteome Research,* **9** (2): 1182-1190.

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Trypanosoma brucei causes African trypanosomiasis in humans (HAT or African sleeping sickness) and nagana in cattle. The disease threatens over 60 million people and uncounted numbers of cattle in 36 countries of sub-Saharan Africa and has a devastating impact on human health and the economy. On the other hand, *Trypanosoma cruzi* is responsible in South America for Chagas disease, which can cause acute illness and death, especially in young children. In this context, the discovery of novel drug targets in the trypanosome proteome is a major focus for the scientific community. Recently, many researchers have spent important efforts on the study of protein-protein interactions (PPIs) in pathogenic trypanosome species concluding that the low sequence identities between some parasite proteins and their human host render these PPIs as highly promising drug targets. To the best of our knowledge, there are no general models to predict unique PPIs in trypanosomes (TPPIs). On the other hand, the 3D structure of an increasing number of trypanosome proteins is reported in databases. In this regard, the introduction of a new model to predict TPPIs from the 3D structure of proteins involved in PPI is very important. For this purpose, we introduced new protein-protein complex invariants based on the Markov average electrostatic potential for amino acids located in different regions (R_i) of i^{th} protein and

placed at a distance k one from each other. We calculated more than 30 different types of parameters for 7 866 pairs of proteins (1 023 TPPIs and 6 823 non-TPPIs) from more than 20 organisms, including parasites and human or cattle hosts. We found a very simple linear model that predicts more than 90 percent of TPPIs and non-TPPIs both in training and independent test subsets using only two parameters. We also tested nonlinear ANN models for comparison purposes but the linear model gives the best results. We implemented this predictor in the web server named TrypanoPPI which is freely available to the public at http://miaja.tic.udc.es/Bio-AIMS/TrypanoPPI.php. This is the first model that predicts how unique a protein-protein complex in trypanosome proteome is with respect to other parasites and hosts, opening new opportunities for antitrypanosome drug target discovery.

15327. **Ruda, G. F., Campbell, G., Alibu, V. P., Barrett, M. P., Brenk, R. & Gilbert, I. H., 2010.** Virtual fragment screening for novel inhibitors of 6-phosphogluconate dehydrogenase. *Bioorganic & Medicinal Chemistry,* **18** (14): 5056-5062.

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The enzyme 6-phosphogluconate dehydrogenase is a potential drug target for the parasitic protozoan *Trypanosoma brucei*, the causative organism of human African trypanosomiasis. This enzyme has a polar active site to accommodate the phosphate, hydroxyl and carboxylate groups of the substrate, 6-phosphogluconate. A virtual fragment screen was undertaken of the enzyme to discover starting points for the development of inhibitors which are likely to have appropriate physicochemical properties for an orally bioavailable compound. A virtual screening library was developed, consisting of compounds with functional groups that could mimic the phosphate group of the substrate, but which have a higher pK_a. Following docking, hits were clustered and appropriate compounds purchased and assayed against the enzyme. Three fragments were identified that had IC_{50} values in the low µ molar range and good ligand efficiencies. Based on these initial hits, analogues were procured and further active compounds were identified. Some of the fragments identified represent potential starting points for a medicinal chemistry programme to develop potent drug-like inhibitors of the enzyme.

15328. **Sharlow, E. R., Lyda, T. A., Dodson, H. C., Mustata, G., Morris, M. T., Leimgruber, S. S., Lee, K. H., Kashiwada, Y., Close, D., Lazo, J. S. & Morris, J. 2010.** A target-based high throughput screen yields *Trypanosoma brucei* hexokinase small molecule inhibitors with antiparasitic activity. *PLoS Neglected Tropical Diseases,* **4** (4): e659.

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The parasitic protozoan *Trypanosoma brucei* utilizes glycolysis exclusively for ATP production during infection of the mammalian host. The first step in this metabolic pathway is mediated by hexokinase (TbHK), an enzyme essential to the parasite that transfers the gamma-phospho of ATP to a hexose. Here we describe the identification and confirmation of novel small molecule inhibitors of bacterially expressed TbHK1, one of two TbHKs

expressed by *T. brucei*, using a high throughput screening assay. Exploiting optimized high throughput screening assay procedures, we interrogated 220 233 unique compounds and identified 239 active compounds from which ten small molecules were further characterized. Computation chemical cluster analyses indicated that six compounds were structurally related while the remaining four compounds were classified as unrelated or singletons. All ten compounds were approximately 20-17 000-fold more potent than lonidamine, a previously identified TbHK1 inhibitor. Seven compounds inhibited *T. brucei* blood stage form parasite growth ($0.03 < \text{or} = EC_{50} < 3$ µM) with parasite specificity of the compounds being demonstrated using insect stage *T. brucei* parasites, *Leishmania* promastigotes, and mammalian cell lines. Analysis of two structurally related compounds, ebselen and SID 17387000, revealed that both were mixed inhibitors of TbHK1 with respect to ATP. Additionally, both compounds inhibited parasite lysate-derived HK activity. None of the compounds displayed structural similarity to known hexokinase inhibitors or human African trypanosomiasis therapeutics. The novel chemotypes identified here could represent leads for future therapeutic development against the African trypanosome.

15329. **Smithson, D. C., Lee, J., Shelat, A. A., Phillips, M. A. & Guy, R. K., 2010.** Discovery of potent and selective inhibitors of *Trypanosoma brucei* ornithine decarboxylase. *Journal of Biological Chemistry,* **285** (22): 16771-16781.

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Human African trypanosomiasis, caused by the eukaryotic parasite *Trypanosoma brucei*, is a serious health problem in much of central Africa. The only validated molecular target for treatment of human African trypanosomiasis is ornithine decarboxylase (ODC), which catalyzes the first step in polyamine metabolism. Here, we describe the use of an enzymatic high throughput screen of 316 114 unique molecules to identify potent and selective inhibitors of ODC. This screen identified four novel families of ODC inhibitors, including the first inhibitors selective for the parasitic enzyme. These compounds display unique binding modes, suggesting the presence of allosteric regulatory sites on the enzyme. Docking of a subset of these inhibitors, coupled with mutagenesis, also supports the existence of these allosteric sites.

15330. **Sokolova, A. Y., Wyllie, S., Patterson, S., Oza, S. L., Read, K. D. & Fairlamb, A. H., 2010.** Cross-resistance to nitro drugs and implications for treatment of human African trypanosomiasis. *Antimicrobial Agents & Chemotherapy,* **54** (7): 2893-2900.

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The success of nifurtimox-eflornithine combination therapy (NECT) for the treatment of human African trypanosomiasis (HAT) has renewed interest in the potential of nitro drugs as chemotherapeutics. In order to study the implications of the more widespread use of nitro drugs against these parasites, we examined the *in vivo* and *in vitro* resistance potentials of nifurtimox and fexinidazole and its metabolites. Following selection *in vitro* by exposure to

increasing concentrations of nifurtimox, *Trypanosoma brucei brucei* nifurtimox-resistant clones designated NfxR1 and NfxR2 were generated. Both cell lines were found to be 8-fold less sensitive to nifurtimox than parental cells and demonstrated cross-resistance to a number of other nitro drugs, most notably the clinical trial candidate fexinidazole (approximately 27-fold more resistant than parental cells). Studies on mice confirmed that the generation of nifurtimox resistance in these parasites did not compromise virulence, and NfxR1 remained resistant to both nifurtimox and fexinidazole *in vivo*. In the case of fexinidazole, drug metabolism and pharmacokinetic studies indicate that the parent drug is rapidly metabolized to the sulfoxide and sulfone form of this compound. These metabolites retained trypanocidal activity but were less effective in nifurtimox-resistant lines. Significantly, trypanosomes selected for resistance to fexinidazole were 10-fold more resistant to nifurtimox than parental cells. This reciprocal cross-resistance has important implications for the therapeutic use of nifurtimox in a clinical setting and highlights a potential danger in the use of fexinidazole as a monotherapy.

15331. **Spavieri, J., Allmendinger, A., Kaiser, M., Casey, R., Hingley-Wilson, S., Lalvani, A., Guiry, M. D., Blunden, G. & Tasdemir, D., 2010.** Antimycobacterial, antiprotozoal and cytotoxic potential of twenty-one brown algae (Phaeophyceae) from British and Irish waters. *Phytotherapy Research***. e publication ahead of print June 17**.

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In the continuation of our research on seaweeds, crude extracts of 21 brown algae collected from the south coast of England and the west coast of Ireland were screened for *in vitro* trypanocidal, leishmanicidal and antimycobacterial activities. Mammalian stages of a small set of parasitic protozoa; i.e. *Trypanosoma brucei rhodesiense, T. cruzi* and *Leishmania donovani*, and the tubercle bacillus *Mycobacterium tuberculosis* were used as test organisms. The extracts were also evaluated for selectivity by testing on a mammalian cell line (L6 cells). Only four extracts were moderately active against *T. cruzi*, whereas all algal extracts showed significant activity against *T. brucei rhodesiense*, with *Halidrys siliquosa* and *Bifurcaria bifurcata* (Sargassaceae) being the most potent (IC_{50} values 1.2 and 1.9 µg/mL). All algal extracts also displayed leishmanicidal activity, with *H. siliquosa* and *B. bifurcata* again being the most active (IC_{50} values 6.4 and 8.6 µg/mL). When tested against *M. tuberculosis*, only the *B. bifurcata* extract was found to have some antitubercular potential (MIC value 64.0 µg/mL). Only three seaweed extracts, i.e. *H. siliquosa, B. bifurcata* and *Cystoseira tamariscifolia* showed some cytotoxicity. To our knowledge, this is the first study on the antiprotozoal and antimycobacterial activity of brown algae from British and Irish waters.

15332. **Tang, S. C. & Shapiro, T. A., 2010.** Newly identified antibacterial compounds are topoisomerase poisons in African trypanosomes. *Antimicrobial Agents & Chemotherapy,* **54** (2): 620-626.

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Human African trypanosomiasis, caused by the *Trypanosoma brucei* protozoan parasite, is fatal when left untreated. Current therapies are antiquated, and there is a need for new pharmacologic agents against *T. brucei* targets that have no human orthologue. Trypanosomes have a single mitochondrion with a unique mitochondrial DNA, known as kinetoplast DNA (kDNA), a topologically complex network that contains thousands of interlocking circular DNAs, termed minicircles (approximately 1 kb) and maxicircles (approximately 23 kb). Replication of kDNA depends on topoisomerases, enzymes that catalyze reactions that change DNA topology. *T. brucei* has an unusual type IA topoisomerase that is dedicated to kDNA metabolism. This enzyme has no orthologue in humans, and RNA interference (RNAi) studies have shown that it is essential for parasite survival, making it an ideal drug target. In a large chemical library screen, two compounds were recently identified as poisons of bacterial topoisomerase IA. We found that these compounds are trypanocidal in the low μ molar range and that they promote the formation of linearized minicircles covalently bound to protein on the 5' end, consistent with the poisoning of mitochondrial topoisomerase IA. Surprisingly, however, band depletion studies showed that it is topoisomerase IImt, and not topoisomerase IAmt, that is trapped. Both compounds are planar aromatic polycyclic structures that intercalate into and unwind DNA. These findings reinforce the utility of topoisomerase IImt as a target for development of new drugs for African sleeping sickness.

15333. **Tulloch, L. B., Martini, V. P., Iulek, J., Huggan, J. K., Lee, J. H., Gibson, C. L., Smith, T. K., Suckling, C. J. & Hunter, W. N., 2010.** Structure-based design of pteridine reductase inhibitors targeting African sleeping sickness and the leishmaniases. *Journal of Medicinal Chemistry,* **53** (1): 221-229.

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Pteridine reductase (PTR1) is a target for drug development against *Trypanosoma* and *Leishmania* species, parasites that cause serious tropical diseases and for which therapies are inadequate. We adopted a structure-based approach to the design of novel PTR1 inhibitors based on three molecular scaffolds. A series of compounds, most newly synthesized, were identified as inhibitors with PTR1-species specific properties explained by structural differences between the *T. brucei* and *L. major* enzymes. The most potent inhibitors target *T. brucei* PTR1, and two compounds displayed antiparasite activity against the bloodstream form of the parasite. PTR1 contributes to antifolate drug resistance by providing a molecular bypass of dihydrofolate reductase (DHFR) inhibition. Therefore, combining PTR1 and DHFR inhibitors might improve therapeutic efficacy. We tested two new compounds with known DHFR inhibitors. A synergistic effect was observed for one particular combination highlighting the potential of such an approach for treatment of African sleeping sickness.

15334. **Watts, K. R., Ratnam, J., Ang, K. H., Tenney, K., Compton, J. E., McKerrow, J. & Crews, P., 2010**. Assessing the trypanocidal potential of natural and semi-

synthetic diketopiperazines from two deep water marine-derived fungi. *Bioorganic & Medicinal Chemistry,* **18** (7): 2566-2574.

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Human African trypanosomiasis (HAT, commonly known as African sleeping sickness) is categorized as a neglected disease, as it afflicts 50 000 people annually in sub-saharan Africa, and there are few formal programmes in the world focused on drug discovery approaches for this disease. In this study, we examined the crude extracts of two fungal strains (*Aspergillus fumigatus* and *Nectria inventa*) isolated from deep water sediment which provided >99 percent growth inhibition at 1µg/mL of *Trypanosoma brucei*, the causative parasite of HAT. A collection of fifteen natural products was supplemented with six semi-synthetic derivatives and one commercially available compound. Twelve of the compounds, each containing a diketopiperazine core, showed excellent activity against *T. brucei* (IC_{50} = 0.002-40 µM), with selectivity over mammalian cells as great as 20-fold. The trypanocidal diketopiperazines were also tested against two cysteine protease targets Rhodesain and TbCatB, where five compounds showed inhibition activity at concentrations less than 20 µM. A preliminary activity pattern is described and analyzed.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

15335**. Tavares, K. C., Da Silva, A. S., Wolkmer, P., Monteiro, S. G. & Miletti, L. C., 2010.** Cryopreservation of *Trypanosoma evansi* after DEAE-cellulose purification: Evaluation of infective parameters. *Research in Veterinary Science*. **e- publication ahead of print June 7.**

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Cryopreservation is a method of keeping parasites alive in a laboratory. However, this technique may also damage the parasite. Alternatively, parasites may be maintained by *in vitro* culture. Unfortunately, for *Trypanosoma evansi* no effective medium that is able to maintain the parasite for more than 4 months has been described. In this study, we examined the effect of purifying trypomastigote through DEAE-cellulose chromatography before and after cryopreservation, by analyzing the pre-patent period, longevity, parasitaemia, and count of viable parasites. Our results showed a three-times increase in the concentration of viable trypomastigotes in DEAE-purified cryopreserved parasites as compared to non-DEAE-purified cryopreserved parasites. This indicates that DEAE-cellulose chromatography followed by cryopreservation is an effective method for the storage and preservation of *T. evansi*, with the advantage that the stocked parasites will be ready to use in molecular biology procedures.

(b) TAXONOMY; CHARACTERIZATION OF ISOLATES

[See also **33**: 15337, 15350, 15352, 15358, 15359, 15382].

15336. **Adams, E. R., Hamilton, P. B. & Gibson, W. C., 2010.** African trypanosomes: celebrating diversity. *Trends in Parasitology,* **26** (7): 324-328.

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Recent advances in molecular identification techniques and phylogenetic analysis have revealed the presence of previously unidentified tsetse-transmitted trypanosomes in Africa. This is surprising in a comparatively well-known group of pathogens that includes the causative agents of human and animal trypanosomiasis. Despite levels of genetic divergence that warrant taxonomic recognition, only one of these new trypanosomes has been named as a new species; the increased diversity is largely ignored or regarded as an inconvenient complication. Yet, some of these trypanosomes have demonstrated pathogenicity, whereas others are closely related to known pathogens, and might share this trait. We should first acknowledge that these novel trypanosomes exist and then take steps to investigate their host range, pathogenicity to livestock and response to chemotherapy.

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

[See also **33**: 15300, 15306, 15311, 15315, 15328].

15337. **Adams, E. R., Hamilton, P. B., Rodrigues, A. C., Malele, II, Delespaux, V., Teixeira, M. M. & Gibson, W., 2010.** New *Trypanosoma (Duttonella) vivax* genotypes from tsetse flies in East Africa. *Parasitology,* **137** (4): 641-650.

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Salivarian trypanosomes pose a substantial threat to livestock, but their full diversity is not known. To survey trypanosomes carried by tsetse in Tanzania, DNA samples from infected proboscides of *Glossina pallidipes* and *G. swynnertoni* were identified using fluorescent fragment length barcoding (FFLB), which discriminates species by size polymorphisms in multiple regions of the ribosomal RNA locus. FFLB identified the trypanosomes in 65 of 105 (61.9 percent) infected proboscides, revealing 9 mixed infections. Of 7 different FFLB profiles, 2 were similar but not identical to reference West African *Trypanosoma vivax*; 5 other profiles belonged to known species also identified in fly midguts. Phylogenetic analysis of the glycosomal glyceraldehyde phosphate dehydrogenase gene revealed that the Tanzanian *T. vivax* samples fell into 2 distinct groups, both outside the main clade of African and South American *T. vivax*. These new *T. vivax* genotypes were common and widespread in tsetse in Tanzania. The *T. brucei*-like trypanosome previously described from tsetse midguts was also found in 2 proboscides, demonstrating a salivarian transmission route. Investigation of mammalian host range and pathogenicity will reveal the importance of

these new trypanosomes for the epidemiology and control of animal trypanosomiasis in East Africa.

15338. **Aeby, E., Ullu, E., Yepiskoposyan, H., Schimanski, B., Roditi, I., Muhlemann, O. & Schneider, A., 2010.** tRNASec is transcribed by RNA polymerase II in *Trypanosoma brucei* but not in humans. *Nucleic Acids Research*. **Published online May 5.**

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Nuclear-encoded tRNAs are universally transcribed by RNA polymerase III (Pol-III) and contain intragenic promoters. Transcription of vertebrate tRNA(Sec) however requires extragenic promoters similar to Pol-III transcribed U6 snRNA. Here, we present a comparative analysis of tRNA(Sec) transcription in humans and the parasitic protozoa *Trypanosoma brucei*, two evolutionary highly diverged eukaryotes. RNAi-mediated ablation of Pol-II and Pol-III as well as oligo-dT induced transcription termination show that the human tRNA(Sec) is a Pol-III transcript. In *T. brucei* protein-coding genes are polycistronically transcribed by Pol-II and processed by trans-splicing and polyadenylation. tRNA genes are generally clustered in between polycistrons. However, the trypanosomal tRNA(Sec) genes are embedded within a polycistron. Their transcription is sensitive to alpha-amanitin and RNAi-mediated ablation of Pol-II, but not of Pol-III. Ectopic expression of the tRNA(Sec) outside but not inside a polycistron requires an added external promoter. These experiments demonstrate that trypanosomal tRNA(Sec), in contrast to its human counterpart, is transcribed by Pol-II. Synteny analysis shows that in trypanosomatids the tRNA(Sec) gene can be found in two different polycistrons, suggesting that it has evolved twice independently. Moreover, intron-encoded tRNAs are present in a number of eukaryotic genomes indicating that Pol-II transcription of tRNAs may not be restricted to trypanosomatids.

15339. **Alves-Silva, J., Ribeiro, J. M., Van Den Abbeele, J., Attardo, G., Hao, Z., Haines, L. R., Soares, M. B., Berriman, M., Aksoy, S. & Lehane, M. J., 2010.** An insight into the sialome of *Glossina morsitans morsitans*. *BMC Genomics,* **11**: 213.

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Blood feeding evolved independently in worms, arthropods and mammals. Among the adaptations to this peculiar diet, these animals developed an armament of salivary molecules that disarm their host's anti-bleeding defences (haemostasis), inflammatory and immune reactions. Recent sialotranscriptome analyses (from the Greek sialo = saliva) of blood feeding insects and ticks have revealed that the saliva contains hundreds of polypeptides, many unique to their genus or family. Adult tsetse flies feed exclusively on vertebrate blood and are important vectors of human and animal diseases. Thus far, only limited information exists regarding the *Glossina* sialome, or any other fly belonging to the Hippoboscidae. As part of

the effort to sequence the genome of *Glossina morsitans morsitans*, several organ specific, high quality normalized cDNA libraries have been constructed, from which over 20 000 ESTs from an adult salivary gland library were sequenced. These ESTs have been assembled using previously described ESTs from the fat body and midgut libraries of the same fly, thus totaling 62 251 ESTs, which have been assembled into 16 743 clusters (8506 of which had one or more EST from the salivary gland library). Coding sequences were obtained for 2 509 novel proteins, 1 792 of which had at least one EST expressed in the salivary glands. Despite library normalization, 59 transcripts were overrepresented in the salivary library indicating high levels of expression. This work presents a detailed analysis of the salivary protein families identified. Protein expression was confirmed by 2D gel electrophoresis, enzymatic digestion and mass spectrometry. Concurrently, an initial attempt to determine the immunogenic properties of selected salivary proteins was undertaken. The sialome of *G. m. morsitans* contains over 250 proteins that are possibly associated with blood feeding. This set includes alleles of previously described gene products, reveals new evidence that several salivary proteins are multigenic and identifies at least seven new polypeptide families unique to *Glossina*. Most of these proteins have no known function and thus provide a discovery platform for the identification of novel pharmacologically active compounds, innovative vector-based vaccine targets, and immunological markers of vector exposure.

15340. **Atyame Nten, C. M., Sommerer, N., Rofidal, V., Hirtz, C., Rossignol, M., Cuny, G., Peltier, J. B. & Geiger, A., 2010.** Excreted/secreted proteins from trypanosome procyclic strains. *Journal of Biomedical Biotechnology,* **2010**: 212817.

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Trypanosoma secretome was shown to be involved in parasite virulence and is suspected of interfering in parasite life-cycle steps such as establishment in the *Glossina* midgut, metacyclogenesis. Therefore, we attempted to identify the proteins secreted by procyclic strains of *T. brucei gambiense* and *T. brucei brucei*, responsible for human and animal trypanosomiasis, respectively. Using mass spectrometry, 427 and 483 nonredundant proteins were characterized in *T. brucei brucei* and *T. brucei gambiense* secretomes, respectively; 35 percent and 42 percent of the corresponding secretome proteins were specifically secreted by *T. brucei brucei* and *T. brucei gambiense*, respectively, while 279 proteins were common to both subspecies. The proteins were assigned to 12 functional classes. Special attention was paid to the most abundant proteases (14 families) because of their potential implication in the infection process and nutrient supply. The presence of proteins usually secreted via an exosome pathway suggests that this type of process is involved in trypanosome ESP secretion. The overall results provide leads for further research to develop novel tools for blocking trypanosome transmission.

15341. **Bodyl, A., Mackiewicz, P. & Milanowski, R., 2010.** Did trypanosomatid parasites contain a eukaryotic alga-derived plastid in their evolutionary past? *Journal of Parasitology,* **96** (2): 465-475.

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The Trypanosomatidae is closely related to euglenids that harbour plastids acquired from a green alga via secondary endosymbiosis. This discovery led to the idea that trypanosomatid parasites contained a green alga-derived plastid in their evolutionary past, an evolutionary scenario that was criticized based on the rarity of plant/plastid/cyanobacterium-like genes in the completely sequenced genomes of *Trypanosoma* and *Leishmania* species. Because it is difficult to identify such genes, however, their apparent rarity does not preclude a previous plastid endosymbiosis in the Trypanosomatidae. The genome of the plastid-less apicomplexan *Cryptosporidium parvum* preserves only a handful of plant/plastid/cyanobacterium-like genes, suggesting massive loss of plastid genes after elimination of its plastid. Additional support for such wholesale gene loss comes from fucoxanthin-containing dinoflagellates. Trypanosomatid nuclear genomes contain cyanobacterium-, green plant-, and haptophyte alga-derived genes, suggesting that they could have possessed a plastid in their evolutionary past; however, these genes also could represent examples of more typical horizontal gene transfer that did not accompany a plastid endosymbiosis. Thus, the presence of host cell genes that were adapted for use in the plastid would be much stronger evidence for a past plastid endosymbiosis in the Trypanosomatidae. Good examples of such genes are those encoding superoxide dismutases (SODs). Trypanosomatid parasites possess 4 iron-containing SODs, with 2 of them, SODA and SODC, targeted to the mitochondrion. In contrast to SODAs with classical single-domain mitochondrial targeting signals, SODCs carry bipartite pre-sequences composed of a signal peptide, followed by a transit peptide. Interestingly, these N-terminal extensions show striking similarities in length, hydropathy profiles, amino acid composition, and targeting properties to pre-sequences of proteins targeted to eukaryotic alga-derived plastids of euglenids and dinoflagellates. In turn, phylogenetic analyses indicate that SODCs originated from a mitochondrion-targeted SOD via gene duplication and were inherited vertically in the trypanosomatid lineage. These data represent a new kind of evidence for a past plastid endosymbiosis in the Trypanosomatidae, but the nature of this plastid remains unclear. It is usually assumed that the trypanosomatid plastid shared a common origin with that of euglenids, but Delta 4 desaturase phylogenies suggest that it could have originated via an independent, tertiary endosymbiosis involving a haptophyte alga. It is also possible that ancestors of the Trypanosomatidae initially possessed a primary plastid that later was replaced by a secondary or tertiary plastid.

15342**. Brenndorfer, M. & Boshart, M., 2010.** Selection of reference genes for mRNA quantification in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **172** (1): 52-55.

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Internal normalization is an established procedure that is necessary for accurate and reliable quantification of differentially regulated mRNAs. The profound changes of gene expression in parasitic life cycles pose a particular challenge to selection of appropriate reference genes for normalization, most importantly when using quantitative real time PCR (qPCR). Here we use the ranking algorithm implemented in the geNorm application to identify suitable *Trypanosoma brucei* reference genes for comparisons between the bloodstream and procyclic developmental stages and for analysis of mRNA induction by

environmental conditions. For these conditions, the TERT gene is a good choice for valid normalization of qPCR and is clearly superior to some other reference genes reported in the literature. For comparison of other conditions, the ranking algorithm is recommended to verify a reliable and valid normalization that is instrumental to quantitative analysis of gene expression.

15343. **Butikofer, P., Greganova, E., Liu, Y. C., Edwards, I. J., Lehane, M. J. & Acosta-Serrano, A., 2010**. Lipid remodelling of glycosylphosphatidylinositol (GPI) glycoconjugates in procyclic-form trypanosomes: biosynthesis and processing of GPIs revisited. *Biochemical Journal,* **428** (3): 409-418.

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The African trypanosome, *Trypanosoma brucei*, has been used as a model to study the biosynthesis of GPI (glycosylphosphatidylinositol) anchors. In mammalian (bloodstream)-form parasites, diacyl-type GPI precursors are remodelled in their lipid moieties before attachment to variant surface glycoproteins. In contrast, the GPI precursors of insect (procyclic)-form parasites, consisting of lyso-(acyl)PI (inositol-acylated acyl-lyso-phosphatidylinositol) species, remain unaltered before protein attachment. By using a combination of metabolic labelling, cell-free assays and complementary MS analyses, we show in the present study that GPI-anchored glycoconjugates in *T. congolense* procyclic forms initially receive tri-acylated GPI precursors, which are subsequently de-acylated either at the glycerol backbone or on the inositol ring. Chemical and enzymatic treatments of ^{3}H myristate-labelled lipids in combination with ESI-MS/MS (electrospray ionization-tandem MS) and MALDI-QIT-TOF-MS3 (matrix-assisted laser-desorption ionization-quadrupole ion trap-time-of-flight MS) analyses indicate that the structure of the lipid moieties of steady-state GPI lipids from *T. congolense* procyclic forms consists of a mixture of lyso-(acyl)PI, diacyl-PI and diacyl-(acyl)PI species. Interestingly, some of these species are myristoylated at the sn-2 position. To our knowledge, this is the first demonstration of lipid remodelling at the level of protein- or polysaccharide-linked GPI anchors in procyclic-form trypanosomes.

15344. **Chou, S., Jensen, B. C., Parsons, M., Alber, T. & Grundner, C., 2010.** The *Trypanosoma brucei* life cycle switch TbPTP1 is structurally conserved and dephosphorylates the nucleolar protein, NOPP44/46. *Journal of Biological Chemistry*. **In press, corrected proof**.

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Trypanosoma brucei adapts to changing environments as it cycles through arrested and proliferating stages in the human and tsetse fly hosts. Changes in protein tyrosine phosphorylation of several proteins, including NOPP44/46, accompany *T. brucei* development. Moreover, inactivation of *T. brucei* protein tyrosine phosphatase 1 (TbPTP1) triggers differentiation of bloodstream stumpy forms into tsetse procyclic forms through unknown downstream effects. Here, we link these events by showing that NOPP44/46 is a major substrate of TbPTP1. TbPTP1 substrate-trapping mutants selectively enrich NOPP44/46 from procyclic stage cell lysates, and TbPTP1 efficiently and selectively dephosphorylates NOPP44/46 *in vitro*. To provide insights into the mechanism of

NOPP44/46 recognition, we determined the crystal structure of TbPTP1. The TbPTP1 structure, the first of a kinetoplastid PTP, emphasizes the conservation of the protein tyrosine phosphatase (PTP) fold, extending to one of the most diverged eukaryotes. The structure reveals surfaces that may mediate substrate specificity and affords a template for the design of selective inhibitors to interfere with *T. brucei* transmission.

15345**. Cliffe, L. J., Siegel, T. N., Marshall, M., Cross, G. A. & Sabatini, R., 2010.** Two thymidine hydroxylases differentially regulate the formation of glucosylated DNA at regions flanking polymerase II polycistronic transcription units throughout the genome of *Trypanosoma brucei*. *Nucleic Acids Research,* **38** (12): 3923-3935.

Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA. [rsabatini@bmb.uga.edu].

Base J is a hypermodified DNA base localized primarily to telomeric regions of the genome of *Trypanosoma brucei*. We have previously characterized two thymidine-hydroxylases (TH), JBP1 and JBP2, which regulate J-biosynthesis. JBP2 is a chromatin re-modelling protein that induces *de novo* J-synthesis, allowing JBP1, a J-DNA binding protein, to stimulate additional J-synthesis. Here, we show that both JBP2 and JBP1 are capable of stimulating *de novo* J-synthesis. We localized the JBP1- and JBP2-stimulated J by anti-J immunoprecipitation and high-throughput sequencing. This genome-wide analysis revealed an enrichment of base J at regions flanking polymerase II polycistronic transcription units (Pol II PTUs) throughout the *T. brucei* genome. Chromosome-internal J deposition is primarily mediated by JBP1, whereas JBP2 stimulated J deposition at the telomeric regions. However, the maintenance of J at JBP1-specific regions is dependent on JBP2 SWI/SNF and TH activity. That similar regions of *Leishmania major* also contain base J highlights the functional importance of the modified base at Pol II PTUs within members of the kinetoplastid family. The regulation of J synthesis/localization by two THs and potential biological function of J in regulating kinetoplastid gene expression are discussed.

15346. **de Jesus, T. C., Tonelli, R. R., Nardelli, S. C., Augusto, L. D., Motta, M. C., Girard-Dias, W., Miranda, K., Ulrich, P., Jimenez, V., Barquilla, A., Navarro, M., Docampo, R. & Schenkman, S., 2010.** Tor-like 1 kinase is involved in the control of polyphosphate levels and acidocalcisome maintenance in *Trypanosoma brucei*. *Journal of Biological Chemistry*. **In press, corrected proof**.

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Target of rapamycin (TOR) kinases are highly conserved protein kinases that integrate signals from nutrients and growth factors to coordinate cell growth and cell cycle progression. It has been previously described that two TOR kinases control cell growth in the protozoan parasite *Trypanosoma brucei*, the causative agent of African trypanosomiasis. Here we studied an unusual TOR-like protein named TbTOR-like 1, containing a PDZ domain and found exclusively in kinetoplastids. TbTOR-like 1 localizes to unique cytosolic granules. After hyperosmotic stress the localization of the protein shifts to the cell periphery, differently from other organelle markers. Ablation of TbTOR-like 1 causes a progressive inhibition of cell proliferation, producing parasites accumulating in S/G2 phase of the cell cycle. TbTOR-like 1 knocked down cells have an increased area occupied by acidic vacuoles,

known as acidocalcisomes, and are enriched in polyphosphate and pyrophosphate. These results suggest that TbTOR-like 1 might be involved in the control of acidocalcisome and polyphosphate metabolism in *T. brucei.*

15347. **de Sousa, K. P., Atouguia, J. & Silva, M. S., 2010.** Partial biochemical characterization of a metalloproteinase from the bloodstream forms of *Trypanosoma brucei brucei* parasites. *Protein Journal,* **29** (4): 283-289.

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Metalloproteinases (MMP) belong to the family of cation dependent endopeptidases that degrade matrices at physiological pH and cleave extracellular matrix proteins. They play an important role in diverse physiological and pathological processes; not only their diverse types of MMP differ in structure and functionally, but also their enzymatic activity is regulated at multiple levels. Trying to shed some light over the processes that govern the pathology of African trypanosomiasis, the aim of the present study was to examine the proteolytic activity of the crude trypanosome protein extract obtained from the bloodstream forms of *Trypanosoma brucei brucei* parasites. We hereby report the partial biochemical characterization of a neutral *Trypanosoma brucei*-metalloproteinase that displays marked proteolytic activities on gelatin and casein, with a molecular mass of approximately 40 kDa, whose activity is strongly dependent of pH and temperature. Furthermore, we show that this activity can be inhibited by classical MMP inhibitors such as EDTA, EGTA, phenantroline, and also by tetracycline and derivatives. This study has a relevant role in the search for new therapeutical targets, for the use of metalloproteinases inhibitors as treatment strategies, or as enhancement to trypanocidal drugs used in the treatment of the disease.

15348**. Denton, H., Fyffe, S. & Smith, T. K., 2010.** GDP-mannose pyrophosphorylase is essential in the bloodstream form of *Trypanosoma brucei*. *Biochemical Journal,* **425** (3): 603-614.

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A putative GDP-Man PP (guanidine diphosphomannose pyrophosphorylase) gene from *Trypanosoma brucei* (TbGDP-Man PP) was identified in the genome and subsequently cloned, sequenced and recombinantly expressed, and shown to be a catalytically active dimer. Kinetic analysis revealed a V_{max} of 0.34 µM/min/ mg of protein and K_m values of 67 µM and 12 µM for GTP and mannose 1-phosphate respectively. Further kinetic studies showed GDP-Man was a potent product feedback inhibitor. RNAi (RNA interference) of the cytosolic TbGDP-Man PP showed that mRNA levels were reduced to ~20 percent of wild-type levels, causing the cells to die after 3-4 days, demonstrating that TbGDP-Man PP is essential in the bloodstream form of *T. brucei* and thus a potential drug target. The RNAi-induced parasites have a greatly reduced capability to form GDP-Man, leading ultimately to a reduction in their ability to synthesize their essential GPI (glycosylphosphatidylinositol) anchors. The RNAi-induced parasites also showed aberrant N-glycosylation of their major cell-surface

glycoprotein, variant surface glycoprotein, with loss of the high-mannose Man9GlcNAc2 N-glycosylation at Asn428 and formation of complex N-glycans at Asn263.

15349. **Erben, E. D., Valguarnera, E., Nardelli, S., Chung, J., Daum, S., Potenza, M., Schenkman, S. & Tellez-Inon, M. T., 2010.** Identification of an atypical peptidyl-prolyl cis/trans isomerase from trypanosomatids. *Biochimica et Biophysica Acta,* **1803** (9): 1028-1037.

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15350. **Fisher, P., Noyes, H., Kemp, S., Stevens, R. & Brass, A., 2009**. A systematic strategy for the discovery of candidate genes responsible for phenotypic variation. *Methods in Molecular Biology,* **573**: 329-345.

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It is increasingly common to combine genome-wide expression data with quantitative trait mapping data to aid in the search for sequence polymorphisms responsible for phenotypic variation. By joining these complex but different data types at the level of the biological pathway, we can take advantage of existing biological knowledge to systematically identify possible mechanisms of genotype-phenotype interaction. With the development of web services and workflows, this process can be made rapid and systematic. Our methodology was applied to a case of resistance to African trypanosomiasis in mice. Workflows developed in this investigation, including a guide to loading and executing them with example data, are available at http://www.myexperiment.org/users/43/workflows.

15351. **Fisk, J. C., Zurita-Lopez, C., Sayegh, J., Tomasello, D. L., Clarke, S. G. & Read, L. K., 2010.** TbPRMT6 is a type I protein arginine methyltransferase that contributes to cytokinesis in *Trypanosoma brucei*. *Eukaryotic Cell,* **9** (6): 866-877.

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Arginine methylation is a widespread posttranslational modification of proteins catalyzed by a family of protein arginine methyltransferases (PRMTs). In *Saccharomyces cerevisiae* and mammals, this modification affects multiple cellular processes, such as chromatin remodelling leading to transcriptional regulation, RNA processing, DNA repair, and cell signalling. The protozoan parasite *Trypanosoma brucei* possesses five putative PRMTs in its genome. This is a large number of PRMTs relative to other unicellular eukaryotes, suggesting an important role for arginine methylation in trypanosomes. Here, we present the *in vitro* and *in vivo* characterization of a *T. brucei* enzyme homologous to human PRMT6, which we term TbPRMT6. Like human PRMT6, TbPRMT6 is a type I PRMT, catalyzing the production of monomethylarginine and asymmetric dimethylarginine residues. In *in vitro* methylation assays, TbPRMT6 utilizes bovine histones as a substrate, but it does not methylate several *T. brucei* glycine/arginine-rich proteins. As such, it exhibits a relatively

narrow substrate specificity compared to other *T. brucei* PRMTs. Knockdown of TbPRMT6 in both procyclic form and bloodstream form *T. brucei* leads to a modest but reproducible effect on parasite growth in culture. Moreover, upon TbPRMT6 depletion, both PF and BF exhibit aberrant morphologies indicating defects in cell division, and these defects differ in the two life cycle stages. Mass spectrometry of TbPRMT6-associated proteins reveals histones, components of the nuclear pore complex, and flagellar proteins that may represent TbPRMT6 substrates contributing to the observed growth and morphological defects.

15352. **Gibson, W., Nemetschke, L. & Ndung'u, J., 2010.** Conserved sequence of the TgsGP gene in Group 1 *Trypanosoma brucei gambiense*. *Infection, Genetics & Evolution,* **10** (4): 453-458.

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The trypanosome responsible for the majority of cases of human trypanosomiasis in Africa is Group 1 *Trypanosoma brucei gambiense*. Currently the most reliable test for the parasite is based on a single gene, which encodes a 47kDa receptor-like *T. b. gambiense*-specific glycoprotein, TgsGP, expressed in the flagellar pocket of bloodstream forms. Although TgsGP has been demonstrated in *T. b. gambiense* throughout its geographic range, similar genes have been demonstrated in other *T. brucei* sspp. isolates, and there are no data on the extent of sequence variation in TgsGP. Here we have carried out a comparison of TgsGP sequences in a range of Group 1 *T. b. gambiense* isolates and compared the gene to homologues in other *T. brucei* sspp. in order to provide information to support the use of this gene as the key identification target for Group 1 *T. b. gambiense*. We demonstrate that the sequence of TgsGP is well conserved in Group 1 *T. b. gambiense* across the endemic range of Gambian human trypanosomiasis and confirm that this gene is a suitable target for specific detection of this parasite. The TgsGp-like genes in some isolates of *T. b. brucei, T. b. rhodesiense* and Group 2 *T. b. gambiense* are closely similar to VSG Tb10.v4.0178, which may be the ancestral gene from which TgsGP was derived.

15353. **Goh, J. Y., Lai, C. Y., Tan, L. C., Yang, D., He, C. Y. & Liou, Y. C., 2010.** Functional characterization of two novel parvulins in *Trypanosoma brucei*. *FEBS Letters,* **584** (13): 2901-2908.

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Parvulins belong to a family of peptidyl-prolyl cis/trans isomerases (PPIases) that catalyze the cis/trans conformations of prolyl-peptidyl bonds. Herein, we characterized two novel parvulins, TbPIN1 and TbPAR42, in *Trypanosoma brucei*. TbPIN1, a 115 amino-acid protein, contains a single PPIase domain but lacks the N-terminal WW domain. Using NMR spectroscopy, TbPIN1 was found to exhibit PPIase activity toward a phosphorylated substrate. Overexpression of TbPIN1 can rescue the impaired temperature-sensitive phenotype in a mutant yeast strain. TbPAR42, containing 383 amino acids, comprises a novel FHA domain at its N terminus and a C-terminal PPIase domain but is a non-Pin1-type PPIase. Functionally, a knockdown of TbPAR42 in its procyclic form results in reduced proliferation rates suggesting an important role in cell growth.

15354. **Gunzl, A., 2010.** The pre-mRNA splicing machinery of trypanosomes: complex or simplified? *Eukaryot Cell*. **Published online ahead of print June 25.**

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Trypanosomatids are early-diverged, protistan parasites of which *Trypanosoma brucei, Trypanosoma cruzi*, and several species of *Leishmania* cause severe, often lethal diseases in humans. To better combat these parasites, their molecular biology has been a research focus for more than three decades and the discovery of spliced leader (SL) trans splicing in *T. brucei* established a key difference between parasites and hosts. In SL trans splicing, the capped 5' terminal region of the small nuclear SL RNA is fused onto the 5' end of each mRNA. This process, in conjunction with polyadenylation, generates individual mRNAs from polycistronic precursors and creates functional mRNA by providing the cap structure. The reaction is a two step transesterification process analogous to intron removal by cis splicing which, in trypanosomatids, is confined to very few pre-mRNAs. Both types of pre-mRNA splicing are carried out by the spliceosome consisting of five U-rich small nuclear (sn)RNAs and, in humans, of up to approximately 170 different proteins. While trypanosomatids possess a full set of spliceosomal U snRNAs, only few splicing factors were identified by standard genome annotation because trypanosomatid amino acid sequences are among the most divergent in the eukaryotic kingdom. This review focuses on recent progress made in the characterization of the splicing factor repertoire in *T. brucei* which was achieved by tandem affinity purification of splicing complexes, by systematic analysis of proteins containing RNA recognition motifs, and by mining the genome database. In addition, recent findings about functional differences between trypanosome and human pre-mRNA splicing factors are discussed.

15355. **Gupta, S. K., Hury, A., Ziporen, Y., Shi, H., Ullu, E. & Michaeli, S., 2010**. Small nucleolar RNA interference in *Trypanosoma brucei*: mechanism and utilization for elucidating the function of snoRNAs. *Nucleic Acids Research*. **Published online July 3**.

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15356. **Hill, K. L., 2010.** Parasites in motion: flagellum-driven cell motility in African trypanosomes. *Current Opinion in Microbiology*, **13** (4): 459-465

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Motility of the sleeping sickness parasite, *Trypanosoma brucei*, impacts disease transmission and pathogenesis. Trypanosome motility is driven by a flagellum that harbours a canonical 9+2 axoneme, together with trypanosome-specific elaborations. Trypanosome flagellum biology and motility have been the object of intense research over the last two years. These studies have led to the discovery of a novel form of motility, termed social motility, and provided revision of long-standing models for cell propulsion. Recent work has also uncovered novel structural features and motor proteins associated with the flagellar apparatus and has identified candidate signalling molecules that are predicted to regulate flagellar motility. Together with earlier inventories of flagellar proteins from proteomic and genomic studies, the stage is now set to move forward with functional studies to elucidate molecular mechanisms and investigate parasite motility in the context of host-parasite interactions.

15357. **Holzmuller, P., Herder, S., Cuny, G. & De Meeus, T., 2010.** From clonal to sexual: a step in *T. congolense* evolution? *Trends in Parasitology,* **26** (2): 56-60.

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Although clearly demonstrated in *Trypanosoma brucei*, genetic exchange remains controversial in other trypanosome species. Recently, Morrison and co-workers applied a population-genetics analysis, and established the existence of mating in *Trypanosoma congolense*. Starting from this original discovery, we focus here on the important question of how mating is induced during the trypanosome life cycle and discuss the use of statistics to evidence this type of non-obligatory biological process.

15358. **Jackson, A. P., Sanders, M., Berry, A., McQuillan, J., Aslett, M. A., Quail, M. A., Chukualim, B., Capewell, P., MacLeod, A., Melville, S. E., Gibson, W., Barry, J. D., Berriman, M. & Hertz-Fowler, C., 2010.** The genome sequence of *Trypanosoma brucei gambiense*, causative agent of chronic human African trypanosomiasis. *PLoS Neglected Tropical Diseases,* **4** (4): e658.

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Trypanosoma brucei gambiense is the causative agent of chronic human African trypanosomiasis or sleeping sickness, a disease endemic across often poor and rural areas of Western and Central Africa. We have previously published the genome sequence of a *T. b. brucei* isolate, and have now employed a comparative genomics approach to understand the scale of genomic variation between *T. b. gambiense* and the reference genome. We sought to identify features that were uniquely associated with *T. b. gambiense* and its ability to infect humans. An improved high-quality draft genome sequence for the group 1 *T. b. gambiense* DAL 972 isolate was produced using a whole-genome shotgun strategy. Comparison with *T. b. brucei* showed that sequence identity averages 99.2 percent in coding regions, and gene order is largely collinear. However, variation associated with segmental duplications and tandem gene arrays suggests some reduction of functional repertoire in *T. b. gambiense* DAL 972. A comparison of the variant surface glycoproteins (VSG) in *T. b. brucei* with all *T. b.*

gambiense sequence reads showed that the essential structural repertoire of VSG domains is conserved across *T. brucei*. This study provides the first estimate of intraspecific genomic variation within *T. brucei*, and so has important consequences for future population genomics studies. We have shown that the *T. b. gambiense* genome corresponds closely with the reference, which should therefore be an effective scaffold for any *T. brucei* genome sequence data. As VSG repertoire is also well conserved, it may be feasible to describe the total diversity of variant antigens. While we describe several as yet uncharacterized gene families with predicted cell surface roles that were expanded in number in *T. b. brucei*, no *T. b. gambiense*-specific gene was identified outside of the subtelomeres that could explain the ability to infect humans.

15359. **Kabani, S., Waterfall, M. & Matthews, K. R., 2010.** Cell-cycle synchronisation of bloodstream forms of *Trypanosoma brucei* using Vybrant DyeCycle Violet-based sorting. *Molecular and Biochemical Parasitology,* **169** (1): 59-62.

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Studies on the cell-cycle of *Trypanosoma brucei* have revealed several unusual characteristics that differ from the model eukaryotic organisms. However, the inability to isolate homogenous populations of parasites in distinct cell-cycle stages has limited the analysis of trypanosome cell division and complicated the understanding of mutant phenotypes with possible impact on cell-cycle related events. Although hydroxyurea-induced cell-cycle arrest in procyclic and bloodstream forms has been applied recently with success, such block-release protocols can complicate the analysis of cell-cycle regulated events and have the potential to disrupt important cell-cycle checkpoints. An alternative approach based on flow cytometry of parasites stained with Vybrant DyeCycle Orange circumvents this problem, but is restricted to procyclic form parasites. Here, we apply Vybrant Dyecycle Violet staining coupled with flow cytometry to effectively select different cell-cycle stages of bloodstream form trypanosomes. Moreover, the sorted parasites remain viable, although synchrony is rapidly lost. This method enables cell-cycle enrichment of populations of trypanosomes in their mammal infective stage, particularly at the G1 phase.

15360. **Kramer, S., Kimblin, N. C. & Carrington, M., 2010.** Genome-wide *in silico* screen for CCCH-type zinc finger proteins of *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania major*. *BMC Genomics,* **11**: 283.

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CCCH type zinc finger proteins are RNA binding proteins with regulatory functions at all stages of mRNA metabolism. The best-characterized member, tritetraproline (TTP), binds to AU rich elements in 3' UTRs of unstable mRNAs, mediating their degradation. In kinetoplastids, CCCH type zinc finger proteins have been identified as being involved in the regulation of the life cycle and possibly the cell cycle. To date, no systematic listing of CCCH proteins in kinetoplastids is available. We have identified the complete set of CCCH type zinc finger proteins in the available genomes of the kinetoplastid protozoa *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania major*. One fifth (20 percent) of all CCCH motifs

fall into non-conventional classes and many had not been previously identified. One third of all CCCH proteins have more than one CCCH motif, suggesting multivalent RNA binding. One third have additional recognizable domains. The vast majority are unique to Kinetoplastida or to a subgroup within. Two exceptions are of interest: the putative orthologue of the mRNA nuclear export factor Mex67 and a 3'-5' exoribonuclease restricted to *Leishmania* species. CCCH motifs are absent from these proteins in other organisms and might be unique, novel features of the Kinetoplastida homologues. Of the others, several have a predicted, and in one case experimentally confirmed, connection to the ubiquitination pathways, for instance a HECT-type E3 ubiquitin ligase. The total number of kinetoplastid CCCH proteins is similar to the number in higher eukaryotes but lower than in yeast. A comparison of the genomic loci between the Trypanosomatidae homologues provides insight into both the evolution of the CCCH proteins as well as the CCCH motifs. This study provides the first systematic listing of the Kinetoplastida CCCH proteins. The number of CCCH proteins with more then one CCCH motif is larger than previously estimated, due to the identification of non-conventional CCCH motifs. Experimental approaches are now necessary to examine the functions of the many unique CCCH proteins as well as the function of the putative Mex67 and the *Leishmania* 3'-5' exoribonuclease.

15361. **Li, Z., Umeyama, T., Li, Z. & Wang, C. C., 2010.** Polo-like kinase guides cytokinesis in *Trypanosoma brucei* through an indirect means. *Eukaryotic Cell,* **9** (5): 705-716.

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Polo-like kinase in *Trypanosoma brucei* (TbPLK) is confined to the flagellum attachment zone (FAZ) and regulates only cytokinetic initiation. However, it apparently diffuses into the cytoplasm before the trans-localization of chromosomal passenger complex (CPC) from the midzone of central spindle to FAZ, which is known to be required for initiating cytokinesis. Synchronized *T. brucei* procyclic cells treated with a TbPLK inhibitor, GW843682X (GW), in late S phase were found to go through a full cell cycle at a normal pace before being arrested at cytokinetic initiation in the second cycle. However, synchronized cells treated with GW in G(1) phase were arrested at cytokinetic initiation within the first cell cycle, suggesting that inhibition of TbPLK at its emergence blocks cytokinesis within the same cell cycle. To rule out potential off-target effects from GW, TbPLK RNA interference (RNAi) was induced to deplete TbPLK, and the progression of synchronized cells from late S phase was also found to be arrested at cytokinetic initiation within the first cell cycle. Apparently, TbPLK has accomplished its role in guiding cytokinesis before the late S phase, presumably by phosphorylating a certain substrate(s) during S phase, which may play a critical role in initiating the subsequent cytokinesis.

15362. **Louw, C. A., Ludewig, M. H. & Blatch, G. L., 2010.** Overproduction, purification and characterisation of Tbj1, a novel Type III Hsp40 from *Trypanosoma brucei*, the African sleeping sickness parasite. *Protein Expression and Purification,* **69** (2): 168-177.

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15363. **Lun, Z. R., Lai, D. H., Li, F. J., Lukes, J. & Ayala, F. J., 2010.** *Trypanosoma brucei*: two steps to spread out from Africa. *Trends in Parasitology*. **In press, corrected proof.**

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Trypanosoma brucei equiperdum and *Trypanosoma brucei evansi* are typically considered separate species, although a recent study suggested that these organisms can be classified as subspecies of *Trypanosoma brucei*, which we also favour. Here we present a scenario that attempts to explain the continuing evolution of the dyskinetoplastic and akinetoplastic strains, as a consequence of loss of selective pressure(s) leading to the loss of kinetoplast DNA.

15364. **Ma, J., Benz, C., Grimaldi, R., Stockdale, C., Wyatt, P., Frearson, J. & Hammarton, T. C., 2010.** Nuclear DBF-2-related kinases are essential regulators of cytokinesis in bloodstream stage *Trypanosoma brucei*. *Journal of Biological Chemistry,* **285** (20): 15356-15368.

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Nuclear DBF-2-related (NDR) kinases are essential regulators of cell cycle progression, growth, and development in many organisms and are activated by the binding of an Mps One Binder (MOB) protein partner, autophosphorylation, and phosphorylation by an upstream STE20 family kinase. In the protozoan parasite, *Trypanosoma brucei*, the causative agent of human African trypanosomiasis, the NDR kinase, PK50, is expressed in proliferative life cycle stages and was shown to complement a yeast NDR kinase mutant cell line. However, the function of PK50 and a second NDR kinase, PK53, in *T. brucei* has not been determined to date, although trypanosome MOB1 is known to be essential for cytokinesis, suggesting the NDR kinases may also be involved in this process. Here, we show that specific depletion of PK50 or PK53 from bloodstream stage trypanosomes resulted in the rapid accumulation of cells with two nuclei and two kinetoplasts, indicating that cytokinesis was specifically inhibited. This led to a deregulation of the cell cycle and cell death and provides genetic validation of these kinases as potential novel drug targets for human African trypanosomiasis. Recombinant active PK50 and PK53 were produced and biochemically characterized. Both enzymes autophosphorylated, were able to trans-phosphorylate generic kinase substrates *in vitro*, and were active in the absence of phosphorylation by an upstream kinase. Additionally, both enzymes were active in the absence of MOB1 binding, which was also demonstrated to likely be a feature of the kinases *in vivo*. Biochemical characterization of recombinant PK50 and PK53 has revealed key kinetic differences between them, and the identification of *in*

vitro peptide substrates in this study paves the way for high throughput inhibitor screening of these kinases.

15365. **Macgregor, P. & Matthews, K. R., 2010.** New discoveries in the transmission biology of sleeping sickness parasites: applying the basics. *Journal of Molecular Medicine.* **Published online June 5.**

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The sleeping sickness parasite, *Trypanosoma brucei*, must differentiate in response to the changing environments that it encounters during its complex life cycle. One developmental form, the bloodstream stumpy stage, plays an important role in infection dynamics and transmission of the parasite. Recent advances have shed light on the molecular mechanisms by which these stumpy forms differentiate as they are transmitted from the mammalian host to the insect vector of sleeping sickness, tsetse flies. These molecular advances now provide improved experimental tools for the study of stumpy formation and function within the mammalian bloodstream. They also offer new routes to therapy via high-throughput screens for agents that accelerate parasite development. Here, we shall discuss the recent advances that have been made and the prospects for future research now available.

15366. **Marcoux, V., Wei, G., Tabel, H. & Bull, H. J., 2010.** Characterization of major surface protease homologues of *Trypanosoma congolense*. *Journal of Biomedicine & Biotechnology,* **2010**: 418157.

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Trypanosomes encode a family of proteins known as major surface metalloproteases (MSPs). We have identified six putative MSPs encoded within the partially sequenced *T. congolense* genome. Phylogenic analysis indicates that *T. congolense* MSPs belong to five subfamilies that are conserved among African trypanosome species. Molecular modelling, based on the known structure of *Leishmania major* GP63, reveals subfamily-specific structural variations around the putative active site despite conservation of overall structure, suggesting that each MSP subfamily has evolved to recognize distinct substrates. We have cloned and purified a protein encoding the amino-terminal domain of the *T. congolense* homologue TcoMSP-D (most closely related to *Leishmania* GP63). We detect TcoMSP-D in the serum of *T. congolense*-infected mice. Mice immunized with the amino-terminal domain of TcoMSP-D generate a persisting IgG_1 antibody response. Surprisingly, a low-dose challenge of immunized mice with *T. congolense* significantly increases susceptibility to infection, indicating that immunity to TcoMSP-D is a factor affecting virulence.

15367**. Martinez-Calvillo, S., Vizuet-de-Rueda, J. C., Florencio-Martinez, L. E., Manning-Cela, R. G. & Figueroa-Angulo, E. E., 2010.** Gene expression in trypanosomatid parasites. *Journal of Biomedicine and Biotechnology,* **2010**: 525241.

Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonoma de Mexico, Av. De los Barrios 1, Col. Los Reyes Iztacala, Tlalnepantla, Edo. de Mexico, CP 54090, Mexico. [scalv@campus.iztacala.unam.mx].

The parasites *Leishmania* spp., *Trypanosoma brucei*, and *Trypanosoma cruzi* are the trypanosomatid protozoa that cause the deadly human diseases leishmaniasis, African sleeping sickness, and Chagas disease, respectively. These organisms possess unique mechanisms for gene expression such as constitutive polycistronic transcription of protein-coding genes and trans-splicing. Little is known about either the DNA sequences or the proteins that are involved in the initiation and termination of transcription in trypanosomatids. *In silico* analyses of the genome databases of these parasites led to the identification of a small number of proteins involved in gene expression. However, functional studies have revealed that trypanosomatids have more general transcription factors than originally estimated. Many posttranslational histone modifications, histone variants, and chromatin modifying enzymes have been identified in trypanosomatids, and recent genome-wide studies showed that epigenetic regulation might play a very important role in gene expression in this group of parasites. Here, we review and comment on the most recent findings related to transcription initiation and termination in trypanosomatid protozoa.

15368. **Mehlert, A., Sullivan, L. & Ferguson, M. A., 2010.** Glycotyping of *Trypanosoma brucei* variant surface glycoprotein MITat1.8. *Molecular & Biochemical Parasitology*. **In press, corrected proof.**

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Following a switch from variant surface glycoprotein MITat1.4 to variant surface glycoprotein MITat1.8 expression by Lister strain 427 *Trypanosoma brucei brucei* parasites, the latter uncharacterized variant surface glycoprotein was analyzed. Variant surface glycoprotein MITat1.8 was found to be a disulphide-linked homodimer, containing a complex N-linked glycan at Asn58 and a glycosylphosphatidylinositol membrane anchor attached to Asp419. Mass spectrometric analyses demonstrated that the N-glycan is exclusively Galbeta1-4GlcNAcbeta1-2Manalpha1-3(Galbeta1-4GlcNAcbeta1-2Manalpha1-6)Manbeta1-4G lcNAcbeta1-4GlcNAc and that the conserved Man(3)GlcN-myo-inositol glycosylphosphatidylinositol anchor glycan core is substituted with an average of 4 hexose, most likely galactose, residues. The presence of a complex N-glycan at Asn58 is consistent with the relatively acidic environment of the Asn58 N-glycosylation sequon, that predicts N-glycosylation by *T. brucei* oligosaccharyltransferase TbSTT3A with a Man(5)GlcNAc(2) structure destined for processing to a paucimannose and/or complex N-glycan.

15369. **Mohd Ismail, N. I., Yuasa, T., Yuasa, K., Nambu, Y., Nisimoto, M., Goto, M., Matsuki, H., Inoue, M., Nagahama, M. & Tsuji, A., 2010.** A critical role for highly conserved Glu(610) residue of oligopeptidase B from *Trypanosoma brucei* in thermal stability. *Journal of Biochemistry*, **147** (2): 201-211.

Department of Biological Science and Technology, University of Tokushima Graduate School, 2-1 Minamijosanjima, Tokushima 770-8506, Japan. [tsuji@bio.tokushima-u.ac.jp].

15370. **Mosimann, M., Goshima, S., Wenzler, T., Luscher, A., Uozumi, N. & Maser, P., 2010.** A Trk/HKT-type K+ transporter from *Trypanosoma brucei*. *Eukaryotic Cell,* **9** (4): 539-546.

Institute of Cell Biology, University of Bern, Bern, Switzerland. [pascal.maeser@unibas.ch].

15371. **Nganga, J. K., Soller, M. & Iraqi, F. A., 2010.** High resolution mapping of trypanosomosis resistance loci Tir2 and Tir3 using F12 advanced intercross lines with major locus Tir1 fixed for the susceptible allele. *BMC Genomics,* **11**: 394.

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Trypanosomosis is the most economically important disease constraint to livestock productivity in Africa. A number of trypanotolerant cattle breeds are found in West Africa, and identification of the genes conferring trypanotolerance could lead to effective means of genetic selection for trypanotolerance. In this context, high resolution mapping in mouse models are a promising approach to identifying the genes associated with trypanotolerance. In previous studies, using F2 C57BL/6J x A/J and C57BL/6J x BALB/cJ mouse resource populations, trypanotolerance QTL were mapped within a large genomic intervals of 20-40 cM to chromosomes MMU17, 5 and 1, and denoted Tir1, Tir2 and Tir3 respectively. Subsequently, using F6 C57BL/6J x A/J and C57BL/6J x BALB/cJ F6 advanced intercross lines (AIL), Tir1 was fine mapped to a confidence interval (CI) of less than 1 cM, while Tir2 and Tir3, were mapped within 5-12 cM. Tir1 represents the major trypanotolerance QTL. In order to improve map resolutions of Tir2 and Tir3, an F12 C57BL/6J x A/J AIL population fixed for the susceptible alleles at Tir1 QTL was generated. An F12 C57BL/6J x A/J AIL population, fixed for the resistant alleles at Tir1 QTL was also generated to provide an additional estimate of the gene effect of Tir1. The AIL populations homozygous for the resistant and susceptible Tir1 alleles and the parental controls were challenged with *T. congolense* and followed for survival times over 180 days. Mice from the two survival extremes of the F12 AIL population fixed for the susceptible alleles at Tir1 were genotyped with a dense panel of microsatellite markers spanning the Tir2 and Tir3 genomic regions and QTL mapping was performed. Tir2 was fine mapped to less than 1 cM CI while Tir3 was mapped to three intervals named Tir3a, Tir3b and Tir3c with 95 percent confidence intervals (CI) of 6, 7.2 and 2.2 cM, respectively. The mapped QTL regions encompass genes that are vital to innate immune response and can be potential candidate genes for the underlying QTL.

15372. **Paris, Z., Changmai, P., Rubio, M. A., Zikova, A., Stuart, K. D., Alfonzo, J. D. & Lukes, J.,** 2010. The Fe/S cluster assembly protein Isd11 is essential for tRNA thiolation in *Trypanosoma brucei*. *Journal of Biological Chemistry,* **285** (29): July 16.

Institute of Parasitology, Czech Republic. [jula@paru.cas.cz].

15373. **Pillay, D., Boulange, A. F. & Coetzer, T. H., 2010.** Expression, purification and characterisation of two variant cysteine peptidases from *Trypanosoma congolense* with active site substitutions. *Protein Expression & Purification*. **In press, corrected proof.**

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Congopain, the major cysteine peptidase of *Trypanosoma congolense* is an attractive candidate for an anti-disease vaccine and target for the design of specific inhibitors. A complicating factor for the inclusion of congopain in a vaccine is that multiple variants of congopain are present in the genome of the parasite. In order to determine whether the variant congopain-like genes code for peptidases with enzymatic activities different to those of congopain, two variants were cloned and expressed. Two truncated catalytic domain variants were recombinantly expressed in *Pichia pastoris*. The two expressed catalytic domain variants differed slightly from one another in substrate preferences and also from that of C2 (the recombinant truncated form of congopain). Surprisingly, a variant with the catalytic triad Ser(25), His(159) and Asn(175) was shown to be active against classical cysteine peptidase substrates and inhibited by E-64, a class specific cysteine protease inhibitor. Both catalytic domain clones and C2 had pH optima of either 6.0 or 6.5 implying that these congopain-like proteases are likely to be expressed and active in the bloodstream of the host animal.

15374. **Price, H. P., Guther, M. L., Ferguson, M. A. & Smith, D. F., 2010.** Myristoyl-CoA:protein N-myristoyltransferase depletion in trypanosomes causes avirulence and endocytic defects. *Molecular & Biochemical Parasitology,* **169** (1): 55-58.

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The enzyme myristoyl-CoA:protein N-myristoyltransferase (NMT) catalyses the co-translational covalent attachment of the fatty acid myristate to the N-terminus of target proteins. NMT is known to be essential for viability in *Trypanosoma brucei* and *Leishmania major*. Here we describe phenotypic analysis of *T. brucei* bloodstream form cells following knockdown of NMT expression by tetracycline-inducible RNA interference. Cell death occurs from 72h post-induction, with approximately 50 percent of cells displaying a defect in endocytic uptake by this time. The majority of these induced cells do not have an enlarged flagellar pocket typical of a block in endocytosis but vesicle accumulation around the flagellar pocket indicates a defect in vesicular progression following endocytic fusion. Induced parasites have a wild-type or slightly enlarged Golgi apparatus, unlike the phenotype of cells with reduced expression of a major N-myristoylated protein, ARL1. Critically we show that following NMT knockdown, *T. brucei* bloodstream form cells are unable to establish an infection in a mouse model, therefore providing further validation of this enzyme as a target for drug development.

15375. **Richmond, G. S., Gibellini, F., Young, S. A., Major, L., Denton, H., Lilley, A. & Smith, T. K., 2010**. Lipidomic analysis of bloodstream and procyclic form *Trypanosoma brucei*. *Parasitology,* **137** (9): 1357-1392.

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The biological membranes of *Trypanosoma brucei* contain a complex array of phospholipids that are synthesized *de novo* from precursors obtained either directly from the host, or as catabolized endocytosed lipids. This paper describes the use of nanoflow electrospray tandem mass spectrometry and high resolution mass spectrometry in both positive and negative ion modes, allowing the identification of approximately 500 individual molecular phospholipids species from total lipid extracts of cultured bloodstream and procyclic form *T. brucei*. Various molecular species of all of the major subclasses of glycerophospholipids were identified including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol as well as phosphatidic acid, phosphatidylglycerol and cardolipin, and the sphingolipids sphingomyelin, inositol phosphoceramide and ethanolamine phosphoceramide. The lipidomic data obtained in this study will aid future biochemical phenotyping of either genetically or chemically manipulated commonly used bloodstream and procyclic strains of *Trypanosoma brucei.* Hopefully this will allow a greater understanding of the bizarre world of lipids in this important human pathogen.

15376. **Richterova, L., Vavrova, Z. & Lukes, J., 2010.** DEAD-box RNA helicase is dispensable for mitochondrial translation in *Trypanosoma brucei*. *Experimental Parasitology*. **In press, corrected proof.**

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, and Faculty of Sciences, University of South Bohemia, Ceske Budejovice (Budweis), Czech Republic. [jula@paru.cas.cz].

15377. **Roy, N., Nageshan, R. K., Pallavi, R., Chakravarthy, H., Chandran, S., Kumar, R., Gupta, A. K., Singh, R. K., Yadav, S. C. & Tatu, U., 2010.** Proteomics of *Trypanosoma evansi* infection in rodents. *PLoS One,* **5** (3): e9796.

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Trypanosoma evansi infections, commonly called “surra”, cause significant economic losses to the livestock industry. While this infection is mainly restricted to large animals such as camels, donkeys and equines, recent reports indicate their ability to infect humans. There are no World Animal Health Organization (WAHO) prescribed diagnostic tests or vaccines available against this disease and the available drugs show significant toxicity. There is an urgent need to develop improved methods of diagnosis and control measures for this disease. Unlike its related human parasites *T. brucei* and *T. cruzi* whose genomes have been fully sequenced, the *T. evansi* genome sequence remains unavailable and very little effort is being made to develop improved methods of prevention, diagnosis and treatment. With a view to identifying potential diagnostic markers and drug targets we have studied the clinical proteome of *T. evansi* infection using mass spectrometry (MS). Using shot-gun proteomic approach involving nano-lc Quadrupole Time Of Flight (QTOF) mass spectrometry we have identified over 160 proteins expressed by *T. evansi* in mice infected with a camel isolate.

Homology driven searches for protein identification from MS/MS data led to most of the matches arising from related *Trypanosoma* species. Proteins identified belonged to various functional categories including metabolic enzymes; DNA metabolism; transcription; translation as well as cell-cell communication and signal transduction. TCA cycle enzymes were strikingly missing, possibly suggesting their low abundances. The clinical proteome revealed the presence of known and potential drug targets such as oligopeptidases, kinases, cysteine proteases and more. Previous proteomic studies on trypanosomal infections, including human parasites *T. brucei* and *T. cruzi*, have been carried out from lab-grown cultures. For *T. evansi* infection this is indeed the first ever proteomic study reported thus far. In addition to providing a glimpse into the biology of this neglected disease, our study is the first step towards identification of diagnostic biomarkers, novel drug targets as well as potential vaccine candidates to fight against *T. evansi* infections.

15378. **Sevova, E. S., Goren, M. A., Schwartz, K. J., Hsu, F. F., Turk, J., Fox, B. G. & Bangs, J. D., 2010.** Cell-free synthesis and functional characterization of sphingolipid synthases from parasitic trypanosomatid protozoa. *Journal of Biological Chemistry*, **285** (27): 20580-20587.

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The *Trypanosoma brucei* genome has four highly similar genes encoding sphingolipid synthases (TbSLS1-4). TbSLSs are polytopic membrane proteins that are essential for viability of the pathogenic bloodstream stage of this human protozoan parasite and, consequently, can be considered as potential drug targets. TbSLS4 was shown previously to be a bifunctional sphingomyelin/ethanolamine phosphorylceramide synthase, whereas functions of the others were not characterized. Using a recently described liposome-supplemented cell-free synthesis system which eliminates complications from background cellular activities, we now unambiguously define the enzymatic specificity of the entire gene family. TbSLS1 produces inositol phosphorylceramide, TbSLS2 produces ethanolamine phosphorylceramide, and TbSLS3 is bifunctional, like TbSLS4. These findings indicate that TbSLS1 is uniquely responsible for synthesis of inositol phosphorylceramide in insect stage parasites, in agreement with published expression array data. This approach also revealed that the *Trypanosoma cruzi* orthologue (TcSLS1) is a dedicated inositol phosphorylceramide synthase. The cell-free synthesis system allowed rapid optimization of the reaction conditions for these enzymes and site-specific mutagenesis to alter end product specificity. A single residue at position 252 (TbSLS1, Ser(252); TbSLS3, Phe(252)) strongly influences enzymatic specificity. We also have used this system to demonstrate that aureobasidin A, a potent inhibitor of fungal inositol phosphorylceramide synthases, does not significantly affect any of the TbSLS activities, consistent with the phylogenetic distance of these two clades of sphingolipid synthases. These results represent the first application of cell-free synthesis for the rapid preparation and functional annotation of integral membrane proteins and thus illustrate its utility in studying otherwise intractable enzyme systems.

15379**. Siegel, T. N., Hekstra, D. R., Wang, X., Dewell, S. & Cross, G. A., 2010.** Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei*

and identification of splicing and polyadenylation sites. *Nucleic Acids Research*. **Published online April 12**.

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Transcription of protein-coding genes in trypanosomes is polycistronic and gene expression is primarily regulated by post-transcriptional mechanisms. Sequence motifs in the untranslated regions regulate mRNA trans-splicing and RNA stability, yet where UTRs begin and end is known for very few genes. We used high-throughput RNA-sequencing to determine the genome-wide steady-state mRNA levels ("transcriptomes") for approximately 90 percent of the genome in two stages of the *Trypanosoma brucei* life cycle cultured *in vitro*. Almost 6 percent of genes were differentially expressed between the two life-cycle stages. We identified 5' splice-acceptor sites (SAS) and polyadenylation sites (PAS) for 6959 and 5948 genes, respectively. Most genes have between one and three alternative SAS, but PAS are more dispersed. For 488 genes, SAS were identified downstream of the originally assigned initiator ATG, so a subsequent in-frame ATG presumably designates the start of the true coding sequence. In some cases, alternative SAS would give rise to mRNAs encoding proteins with different N-terminal sequences. We could identify the introns in two genes known to contain them, but found no additional genes with introns. Our study demonstrates the usefulness of the RNA-sequencing technology to study the transcriptional landscape of an organism whose genome has not been fully annotated.

15380. **Sienkiewicz, N., Ong, H. B. & Fairlamb, A. H., 2010**. *Trypanosoma brucei* pteridine reductase 1 is essential for survival *in vitro* and for virulence in mice. *Molecular Microbiology,* **77** (3): 658-671.

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Gene knockout and knockdown methods were used to examine essentiality of pteridine reductase (PTR1) in pterin metabolism in the African trypanosome. Attempts to generate PTR1 null mutants in bloodstream form *T. brucei* proved unsuccessful; despite integration of drug selectable markers at the target locus, the gene for PTR1 was either retained at the same locus or elsewhere in the genome. However, RNA interference (RNAi) resulted in complete knockdown of endogenous protein after 48 h, followed by cell death after 4 days. This lethal phenotype was reversed by expression of enzymatically active *Leishmania major* PTR1 in RNAi lines or by addition of tetrahydrobiopterin to cultures. Loss of PTR1 was associated with gross morphological changes due to a defect in cytokinesis, resulting in cells with multiple nuclei and kinetoplasts, as well as multiple detached flagella. Electron microscopy also revealed increased numbers of glycosomes, while immunofluorescence microscopy showed increased and more diffuse staining for glycosomal matrix enzymes, indicative of mis-localisation to the cytosol. Mis-localization was confirmed by digitonin fractionation experiments. RNAi cell lines were markedly less virulent than wild-type (WT) parasites in mice and virulence was restored in the (oe)RNAi line. Thus, PTR1 may be a drug target for human African trypanosomiasis.

15381. **Simo, G., Herder, S., Cuny, G. & Hoheisel, J., 2010.** Identification of subspecies specific genes differentially expressed in procyclic forms of *Trypanosoma brucei* subspecies. *Infection, Genetics & Evolution,* **10** (2): 229-237.

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Trypanosoma brucei subspecies undergo establishment and maturation in tsetse flies mid-gut and salivary glands, respectively. Successful establishment of trypanosomes in tsetse mid-gut as well as their migration to saliva gland depends on the ability of these parasites to adapt rapidly to new environmental conditions and to negotiate the physical barriers. To identify subspecies specific genes which are differentially regulated during the establishment of *T. brucei* subspecies in tsetse flies mid-gut, a comparative genomic analysis between different *T. brucei* subspecies was performed using microarrays containing about 23 040 *T. brucei* shotgun fragments. The whole genome analyses of RNA expression profiles revealed about 274 significantly differentially expressed genes between *T. brucei* subspecies. About 7 percent of the differentially regulated clones did not match to any *T. brucei* predicted genes. Most of the differentially regulated transcripts are involved in transport across cell membrane and also in the purines metabolism. The genes selectively up regulated in *T. brucei gambiense* and *T. brucei rhodesiense* (human infective *T. brucei*) like snoRNA and HSP70 are expressed in response to stress. The high failure rate in the process of establishment and maturation of *T. brucei gambiense* during cyclical transmission in tsetse flies may result from the incapacity of this parasite to regulate its growth due to the expression of a variety of chaperones or heat shock proteins. Genes selectively up regulated in *T. brucei brucei* like NT8.1 nucleoside/nucleobase transporters and S-adenosylmethionine synthetase may favour the establishment of this subspecies in tsetse mid-gut. These genes appear as potential targets for investigations on the development of vaccine blocking the transmission of trypanosomes in tsetse flies.

15382. **Simo, G., Njiokou, F., Tume, C., Lueong, S., De Meeus, T., Cuny, G. & Asonganyi, T., 2010**. Population genetic structure of Central African *Trypanosoma brucei gambiense* isolates using microsatellite DNA markers. *Infection, Genetics & Evolution,* **10** (1): 68-76.

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Genetic variation of microsatellite loci is a widely used method for the analysis of population genetic structure of microorganisms. Seven microsatellite markers were used here to characterize *Trypanosoma brucei gambiense* isolates from Central Africa sub-region in order to improve knowledge on the population genetic structure of this subspecies. These markers confirmed the low genetic polymorphism within Central African *T. b. gambiense* isolates from the same focus and strong differentiation between different foci. The presence of many multilocus genotypes of *T. b. gambiense* and the excess of heterozygotes found in this study play in favour of a clonal reproduction of this parasite. But some data may be indicative of a unique recombination event in one subsample. The high F_{ST} value indicates

low migration rates between *T. b. gambiense* subpopulations (foci). Very negative F_{IS} suggests fairly small clonal population sizes of this pathogen in the different human trypanosomosis foci of Central Africa.

15383. **Smith, T. K. & Butikofer, P., 2010.** Lipid metabolism in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **172** (2): 66-79.

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Trypanosoma brucei membranes consist of all major eukaryotic glycerophospholipid and sphingolipid classes. These are *de novo* synthesized from precursors obtained either from the host or from catabolised endocytosed lipids. In recent years, substantial progress has been made in the molecular and biochemical characterization of several of these lipid biosynthetic pathways, using gene knockout or RNA interference strategies or by enzymatic characterization of individual reactions. Together with the completed genome, these studies have highlighted several possible differences between mammalian and trypanosome lipid biosynthesis that could be exploited for the development of drugs against the diseases caused by these parasites.

15384. **Sprehe, M., Fisk, J. C., McEvoy, S. M., Read, L. K. & Schumacher, M. A., 2010.** Structure of the *Trypanosoma brucei* p22 protein, a cytochrome oxidase subunit II-specific RNA-editing accessory factor. *Journal of Biological Chemistry,* **285** (24): 18899-18908.

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15385. **Stagno, J., Aphasizheva, I., Bruystens, J., Luecke, H. & Aphasizhev, R., 2010.** Structure of the mitochondrial editosome-like complex associated TUTase 1 reveals divergent mechanisms of UTP selection and domain organization. *Journal of Molecular Biology,* **399** (3): 464-475.

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RNA uridylylation reactions catalyzed by terminal uridylyl transferases (TUTases) play critical roles in the formation of the mitochondrial transcriptome in trypanosomes. Two mitochondrial RNA editing TUTases have been described: RNA editing TUTase 1 catalyzes guide RNA, ribosomal RNA, and mRNA 3'-uridylylation, and RNA editing TUTase 2 acts as a subunit of the RNA editing core complex (also referred to as the 20S editosome) to perform guided U-insertion mRNA editing. Although RNA editing TUTase 1 and RNA editing TUTase 2 carry out distinct functions and possess dissimilar enzymatic properties, their catalytic N-terminal domain and base recognition C-terminal domain display a high degree of similarity, while their middle domains are less conserved. MEAT1 (mitochondrial editosome-like complex associated TUTase 1), which interacts with an editosome-like assembly and is

exclusively U-specific, nonetheless shows limited similarity with editing TUTases and lacks the middle domain. The crystal structures of apo MEAT1 and UTP-bound MEAT1 refined to 1.56 A and 1.95 A, respectively, reveal an unusual mechanism of UTP selection and domain organization previously unseen in TUTases. In addition to established invariant UTP-binding determinants, we have identified and verified critical contributions of MEAT1-specific residues using mutagenesis. Furthermore, MEAT1 possesses a novel bridging domain, which extends from the C-terminal domain and makes hydrophobic contacts with the N-terminal domain, thereby creating a cavity adjacent to the UTP-binding site. Unlike the minimal TUT4 TUTase, MEAT1 shows no appreciable conformational change upon UTP binding and apparently does not require RNA substrate to select a cognate nucleoside triphosphate. Because MEAT1 is essential for the viability of the bloodstream and insect forms of *Trypanosoma brucei*, the unique organization of its active site renders this protein an attractive target for trypanocide development.

15386. **Stewart, M., Haile, S., Jha, B. A., Cristodero, M., Li, C. H. & Clayton, C., 2010.** Processing of a phosphoglycerate kinase reporter mRNA in *Trypanosoma brucei* is not coupled to transcription by RNA polymerase II. *Molecular & Biochemical Parasitology,* **172** (2): 99-106.

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15387. **Subramanya, S., Armah, D. A. & Mensa-Wilmot, K., 2010**. *Trypanosoma brucei:* reduction of GPI-phospholipase C protein during differentiation is dependent on replication of newly transformed cells. *Experimental Parasitology,* **125** (3): 222-229.

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The protozoan parasite *Trypanosoma brucei* lives in the bloodstream of vertebrates or in a tsetse fly. Expression of a GPI-phospholipase C polypeptide (GPI-PLCp) in the parasite is restricted to the bloodstream form. Events controlling the amount of GPI-PLCp expressed during differentiation are not completely understood. Our metabolic "pulse-chase" analysis reveals that GPI-PLCp is stable in bloodstream form. However, during differentiation of bloodstream to insect stage (procyclic) *T. brucei*, translation GPI-PLC mRNA ceases within 8h of initiating transformation. GPI-PLCp is not lost precipitously from newly transformed procyclic trypanosomes. Nascent procyclics contain 400-fold more GPI-PLCp than established insect stage *T. brucei*. Reduction of GPI-PLCp in early-stage procyclics is linked to parasite replication. Sixteen cell divisions are required to reduce the amount of GPI-PLCp in newly differentiated procyclics to levels present in the established procyclic. GPI-PLCp is retained in strains of *T. brucei* that fail to replicate after differentiation of the bloodstream to the procyclic form. Thus, at least two factors control levels of GPI-PLCp during differentiation of bloodstream *T. brucei*; (i) repression of GPI-PLC mRNA translation, and (ii) sustained replication of newly transformed procyclic *T. brucei*. These studies illustrate the importance of repeated cell divisions in controlling the steady-state amount of GPI-PLCp during differentiation of the African trypanosome.

15388**. Swift, R. V. & Amaro, R. E., 2009**. Discovery and design of DNA and RNA ligase inhibitors in infectious microorganisms. *Expert Opinion on Drug Discovery,* **4** (12): 1281-1294.

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Members of the nucleotidyltransferase superfamily known as DNA and RNA ligases carry out the enzymatic process of polynucleotide ligation. These guardians of genomic integrity share a three-step ligation mechanism, as well as common core structural elements. Both DNA and RNA ligases have experienced a surge of recent interest as chemotherapeutic targets for the treatment of a range of diseases, including bacterial infection, cancer, and the diseases caused by the protozoan parasites known as trypanosomes. In this review, we will focus on efforts targeting pathogenic microorganisms; specifically, bacterial NAD^+-dependent DNA ligases, which are promising broad-spectrum antibiotic targets, and ATP-dependent RNA editing ligases from *Trypanosoma brucei*, the species responsible for the devastating neurodegenerative disease, African sleeping sickness. High quality crystal structures of both NAD^+-dependent DNA ligase and the *Trypanosoma brucei* RNA editing ligase have facilitated the development of a number of promising leads. For both targets, further progress will require surmounting permeability issues and improving selectivity and affinity.

15389. **Szoor, B., 2010.** Trypanosomatid protein phosphatases. *Molecular & Biochemical Parasitology,* **173** (2): 53-63.

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Protein phosphorylation is one of the most important post-translational modifications regulating various signalling processes in all known living organisms. In the cell, protein phosphatases and protein kinases play a dynamic antagonistic role, controlling the phosphorylation state of tyrosine (Tyr), serine (Ser) and threonine (Thr) side chains of proteins. The reversible phosphorylation modulates protein function, through initiating conformational changes, which influences protein complex formation, alteration of enzyme activity and changes in protein stability and subcellular localization. These molecular changes affect signalling cascades regulating the cell cycle, differentiation, cell-cell and cell-substrate interactions, cell motility, the immune response, ion-channel and transporter activities, gene transcription, mRNA translation, and basic metabolism. In addition to these processes, in unicellular parasites, like *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania* spp., additional signalling pathways have evolved to enable the survival of parasites in the changing environment of the vector and host organism. In recent years the genome of five trypanosomatid genomes have been sequenced and annotated allowing complete definition of the composition of the trypanosomatid phosphatomes. The very diverse environments involved in the different stages of the kinetoplastids' life cycle might have played a role to develop a set of trypanosomatid-specific phosphatases in addition to orthologues of many

higher eukaryote protein phosphatases present in the kinetoplastid phosphatomes. In spite of their well-described phosphatomes, few trypanosomatid protein phosphatases have been characterized and studied *in vivo*. The aim of this review is to give an up to date scope of the research, which has been carried out on trypanosomatid protein phosphatases.

15390. **Szoor, B., Ruberto, I., Burchmore, R. & Matthews, K. R., 2010.** A novel phosphatase cascade regulates differentiation in *Trypanosoma brucei* via a glycosomal signalling pathway. *Genes and Development,* **24** (12): 1306-1316.

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In the mammalian bloodstream, the sleeping sickness parasite *Trypanosoma brucei* is held poised for transmission by the activity of a tyrosine phosphatase, TbPTP1. This prevents differentiation of the transmissible "stumpy forms" until entry into the tsetse fly, whereupon TbPTP1 is inactivated and major changes in parasite physiology are initiated to allow colonization of the arthropod vector. Using a substrate-trapping approach, we identified the downstream step in this developmental signalling pathway as a DxDxT phosphatase, TbPIP39, which is activated upon tyrosine phosphorylation, and hence is negatively regulated by TbPTP1. *In vitro*, TbPIP39 promotes the activity of TbPTP1, thereby reinforcing its own repression, this being alleviated by the trypanosome differentiation triggers citrate and cis-aconitate, generating a potentially bistable regulatory switch. Supporting a role in signal transduction, TbPIP39 becomes rapidly tyrosine-phosphorylated during differentiation, and RNAi-mediated transcript ablation in stumpy forms inhibits parasite development. Interestingly, TbPIP39 localizes in glycosomes, peroxisome-like organelles that compartmentalize the trypanosome glycolytic reactions among other enzymatic activities. Our results invoke a phosphatase signalling cascade in which the developmental signal is trafficked to a unique metabolic organelle in the parasite: the glycosome. This is the first characterized environmental signalling pathway targeted directly to a peroxisome-like organelle in any eukaryotic cell.

15391. **Takcz, I. D., Gupta, S. K., Volkov, V., Romano, M., Haham, T., Tulinski, P., Lebenthal, I. & Michaeli, S., 2010.** Analysis of spliceosomal proteins in trypanosomatids reveals novel functions in mRNA processing. *Journal of Biological Chemistry*. **In press, corrected proof.**

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In trypanosomatids, all mRNAs are processed via trans-splicing, though cis-splicing also occurs. In trans-splicing, a common small exon, the spliced leader (SL), which is derived from a small SL RNA species, is added to all mRNAs. Sm and Lsm proteins are core proteins that bind to U snRNAs and are essential for both these splicing processes. In this study, SmD3 and Lsm3 associated complexes were purified to homogeneity from *Leishmania tarentolae.* The purified complexes were analyzed by mass spectrometry and 54 and 39 proteins were purified from SmD3 and Lsm complexes, respectively. Interestingly, among the proteins purified from Lsm3, no mRNA degradation factors were detected, as in Lsm complexes from other eukaryotes. The U1A complex was purified and mass-spectrometry

analysis identified, in addition to U1 snRNP proteins, additional co-purified proteins including the polyadenylation factor, CPSF73. Defects observed in cells silenced for U1 snRNP proteins suggest that the U1 snRNP functions exclusively in cis-splicing, though U1A also participates in polyadenylation and affects trans-splicing. The study characterized several trypanosome-specific nuclear factors involved in snRNP biogenesis, whose function was elucidated in *Trypanosoma brucei*. Conserved factors, such as PRP19, which functions at the heart of every cis-spliceosome, also affects SL RNA modification; GEMIN2, a protein associated with SMN (survival of motor neurons) and implicated in selective association of U snRNA with core Sm proteins in trypanosomes, is a master regulator of snRNP assembly. This study demonstrates the existence of trypanosomatid-specific splicing factors, but also that conserved snRNP proteins possess trypanosome-specific functions.

15392**. Tyc, J., Faktorova, D., Kriegova, E., Jirku, M., Vavrova, Z., Maslov, D. A. & Lukes, J., 2010.** Probing for primary functions of prohibitin in *Trypanosoma brucei*. *International Journal of Parasitology,* **40** (1): 73-83.

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Prohibitins (PHBs) 1 and 2 are small conserved proteins implicated in a number of functions in the mitochondrion, as well as in the nucleus of eukaryotic cells. The current understanding of PHB functions comes from studies of model organisms such as yeast, worm and mouse, but considerable debate remains with regard to the primary functions of these ubiquitous proteins. We exploit the tractable reverse genetics of *Trypanosoma brucei*, the causative agent of African sleeping sickness, in order to specifically analyse the function of PHB in this highly divergent eukaryote. Using inducible RNA interference (RNAi) we show that PHB1 is essential in *T. brucei*, where it is confined to the cell's single mitochondrion forming a high molecular weight complex. PHB1 and PHB2 appear to be indispensible for mitochondrial translation. Their ablation leads to a decrease in mitochondrial membrane potential, however no effect on the level of reactive oxygen species was observed. Flagellates lacking either PHB1 or both PHB1 and PHB2 exhibit significant morphological changes of their organelle, most notably its inflation. Even long after the loss of the PHB proteins, mtDNA was unaltered and mitochondrial cristae remained present, albeit displaced to the periphery of the mitochondrion, which is in contrast to other eukaryotes.

15393. **Tyc, J., Long, S., Jirku, M. & Lukes, J., 2010**. YCF45 protein, usually associated with plastids, is targeted into the mitochondrion of *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **173** (1): 43-47.

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15394. **Vanhollebeke, B., Uzureau, P., Monteyne, D., Perez-Morga, D. & Pays, E., 2010**. Cellular and molecular remodelling of the endocytic pathway during differentiation of *Trypanosoma brucei* bloodstream forms. *Eukaryotic Cell*, **9** (8) 1272-1282.

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During the course of mammalian infection, African trypanosomes undergo extensive cellular differentiation, as actively dividing long slender forms (SL) progressively transform into intermediate forms (I) and finally quiescent G1/G0-locked short stumpy forms (ST). ST forms maintain adaptations compatible with their survival in the mammalian bloodstream such as high endocytic activity, but they already show pre-adaptations to the insect midgut conditions. The nutritional requirements of ST forms must differ from those of SL forms because they do not multiply any longer. We report that in ST forms the uptake of several ligands was reduced as compared with SL forms. In particular, the haptoglobin-haemoglobin (Hp-Hb) complex was no longer taken up due to dramatic down-regulation of its cognate receptor TbHpHbR. As this receptor also allows uptake of trypanolytic particles from human serum, ST forms were resistant to trypanolysis by human serum lipoproteins. These observations allowed both flow cytometry analysis of SL to ST differentiation and the generation of homogeneous ST populations after positive selection upon exposure to trypanolytic particles. In addition, we observed that in ST forms the lysosome relocates anterior to the nucleus. Altogether, we identified novel morphological and molecular features that characterize SL to ST differentiation.

15395. **Vaughan, S., 2010**. Assembly of the flagellum and its role in cell morphogenesis in *Trypanosoma brucei*. *Current Opinion in Microbiology,* **13** (4): 453-458.

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Eukaryotic flagella are microtubule-based structures required for a variety of functions including cell motility and sensory perception. Most eukaryotic flagella grow out from a cell into the surrounding medium, but when the flagellum of the protozoan parasite *Trypanosoma brucei* exits the cell via the flagellar pocket, it is attached along the length of the cell body by a cytoskeletal structure called the flagellum attachment zone (FAZ). The exact reasons for flagellum attachment have remained elusive, but evidence is emerging that the attached flagellum plays a major role in cell morphogenesis in this organism. In this review we discuss evidence published in the past four years that is unravelling the role of the flagellum in organelle segregation, inheritance of cell shape and cytokinesis.

15396. **Veitch, N. J., Johnson, P. C., Trivedi, U., Terry, S., Wildridge, D. & MacLeod, A., 2010.** Digital gene expression analysis of two life cycle stages of the human-infective parasite, *Trypanosoma brucei gambiense* reveals differentially expressed clusters of co-regulated genes. *BMC Genomics,* **11**: 124.

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The evolutionarily ancient parasite, *Trypanosoma brucei*, is unusual in that the majority of its genes are regulated post-transcriptionally, leading to the suggestion that transcript abundance of most genes does not vary significantly between different life cycle stages despite the fact that the parasite undergoes substantial cellular remodelling and metabolic changes throughout its complex life cycle. To investigate this in the clinically relevant sub-species, *Trypanosoma brucei gambiense*, which is the causative agent of the fatal human disease African sleeping sickness, we have compared the transcriptome of two different life cycle stages, the potentially human-infective bloodstream forms with the non-human-infective procyclic stage using digital gene expression (DGE) analysis. Over eleven million unique tags were generated, producing expression data for 7 360 genes, covering 81 percent of the genes in the genome. Compared to microarray analysis of the related *T. b. brucei* parasite, approximately 10 times more genes with a 2.5-fold change in expression levels were detected. The transcriptome analysis revealed the existence of several differentially expressed gene clusters within the genome, indicating that contiguous genes, presumably from the same polycistronic unit, are co-regulated either at the level of transcription or transcript stability. DGE analysis is extremely sensitive for detecting gene expression differences, revealing firstly that a far greater number of genes are stage-regulated than had previously been identified and secondly and more importantly, this analysis has revealed the existence of several differentially expressed clusters of genes present on what appears to be the same polycistronic units, a phenomenon which had not previously been observed in microarray studies. These differentially regulated clusters of genes are in addition to the previously identified RNA polymerase I polycistronic units of variant surface glycoproteins and procyclin expression sites, which encode the major surface proteins of the parasite. This raises a number of questions regarding the function and regulation of the gene clusters that clearly warrant further study.

15397. **Worthen, C., Jensen, B. C. & Parsons, M., 2010**. Diverse effects on mitochondrial and nuclear functions elicited by drugs and genetic knockdowns in bloodstream stage *Trypanosoma brucei. PLoS Neglected Tropical Diseases,* **4** (5): e678.

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The options for treating the fatal disease human African trypanosomiasis are limited to a few drugs that are toxic or facing increasing resistance. New drugs that kill the causative agents, subspecies of *Trypanosoma brucei*, are therefore urgently needed. Little is known about the cellular mechanisms that lead to death of the pathogenic bloodstream stage. We therefore conducted the first side by side comparison of the cellular effects of multiple death inducers that target different systems in bloodstream form parasites, including six drugs (pentamidine, prostaglandin D_2, quercetin, etoposide, camptothecin, and a tetrahydroquinoline) and six RNAi knockdowns that target distinct cellular functions. All compounds tested were static at low concentrations and killed at high concentrations. Dead parasites were rapidly quantified by forward and side scatter during flow cytometry, as confirmed by ethidium homodimer and esterase staining, making these assays convenient for quantitating parasite death. The various treatments yielded different combinations of defects in mitochondrial potential, reactive oxygen species, cell cycle, and genome segregation. No evidence was seen for phosphatidylserine exposure, a marker of apoptosis. Reduction in ATP levels lagged behind decreases in live cell number. Even when the impact on growth was

similar at 24 hours, drug-treated cells showed dramatic differences in their ability to further proliferate, demonstrating differences in the reversibility of effects induced by the diverse compounds. Parasites showed different phenotypes depending on the treatment, but none of them were clear predictors of whether apparently live cells could go on to proliferate after drugs were removed. We therefore suggest that clonal proliferation assays may be a useful step in selecting anti-trypanosomal compounds for further development. Elucidating the genetic or biochemical events initiated by the compounds with the most profound effects on subsequent proliferation may identify new means to activate death pathways.

15398. **Wright, J. R., Siegel, T. N. & Cross, G. A., 2010.** Histone H3 trimethylated at lysine 4 is enriched at probable transcription start sites in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **172** (2): 141-144.

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Recent studies have identified histone modifications and suggested a role for epigenetic gene regulation in *Trypanosoma brucei*. The histone modification H4K10ac and histone variants H2AZ and H2BV localize to probable sites of transcription initiation. Although all *T. brucei* histones have very evolutionarily divergent N-terminal tails, histone H3 shows conservation with other eukaryotic organisms in 6 of 8 amino acids encompassing lysine 4. Tri-methylation of H3K4 is generally associated with transcription. We therefore generated a specific antibody to *T. brucei* H3K4me3 and performed chromosome immunoprecipitation and high-throughput sequencing. We show that H3K4me3 is enriched at the start of polycistronic transcription units at divergent strand-switch regions and at other sites of RNA polymerase II transcription reinitiation. H3K4me3 largely co-localizes with H4K10ac, but with a skew towards the upstream side of the H4K10ac peak, suggesting that it is a component of specific nucleosomes that play a role in Pol II transcription initiation.

15399. **Xia, Y., Zhang, Y., Jiang, S. & Cheng, H., 2010.** CD4(+) T-cell anergy induced by lin(-)CD117(c-kit$^+$) stem cell-derived immature dendritic cells loaded with nuclear antigen derived from *Trypanosoma equiperdum*. *Autoimmunity*. **e publication ahead of print April 7**

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Dendritic cells (DCs) are professional antigen-presenting cells, which have the extraordinary capacity to initiate naive T-cell-mediated primary immune responses. To investigate the role of DCs in the induction of antigen-specific tolerance, the immature DCs (imDCs) and mature DCs (mDCs) were generated *in vitro* from lin(-)CD117(c-kit$^+$) stem cells isolated from mice bone marrow. Flow cytometry and confocal microscopy were used to characterize the phenotypes of DCs. These cells were loaded with nuclear antigen derived from *Trypanosoma equiperdum* and then co-cultured with naive CD4$^+$ T cells. It was found that imDC-treated T cells had lower proliferation level and cytokine expression of interleukin (IL)-2, IL-4, IL-12, and interferon-gamma compared with mDC-treated T cells. These results demonstrated that the maturation status of DCs is critical for preventing the production of autoantibodies.

15400. **Yao, Y., Gao, G. & Li, D., 2010.** Cloning, expression, purification and activity assay of *Trypanosoma brucei* phenylalanyl-tRNA synthetase in *Escherichia coli*. *Sheng Wu Gong Cheng Xue Bao,* **26** (1): 130-135.

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15401. **Young, S. A. & Smith, T. K., 2010.** The essential neutral sphingomyelinase is involved in the trafficking of the variant surface glycoprotein in the bloodstream form of *Trypanosoma brucei*. *Molecular Microbiology,* **76** (6): 1461-1482.

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Sphingomyelin is the main sphingolipid in *Trypanosoma brucei,* the causative agent of African sleeping sickness. *In vitro* and *in vivo* characterization of the *T. brucei* neutral sphingomyelinase demonstrates that it is directly involved in sphingomyelin catabolism. Gene knockout studies in the bloodstream form of the parasite indicate that the neutral sphingomyelinase is essential for growth and survival, thus highlighting that the *de novo* biosynthesis of ceramide is unable to compensate for the loss of sphingomyelin catabolism. The phenotype of the conditional knockout has given new insights into the highly active endocytic and exocytic pathways in the bloodstream form of *T. brucei*. Hence, the formation of ceramide in the endoplasmic reticulum affects post-Golgi sorting and rate of deposition of newly synthesized GPI-anchored variant surface glycoprotein on the cell surface. This directly influences the corresponding rate of endocytosis, via the recycling endosomes, of pre-existing cell surface variant surface glycoprotein. The trypanosomes use this coupled endocytic and exocytic mechanism to maintain the cell density of its crucial variant surface glycoprotein protective coat. TbnSMase is therefore genetically validated as a drug target against African trypanosomes, and suggests that interfering with the endocytic transport of variant surface glycoprotein is a highly desirable strategy for drug development against African trypanosomasis.

15402. **Zhou, Q., Gheiratmand, L., Chen, Y., Lim, T. K., Zhang, J., Li, S., Xia, N., Liu, B., Lin, Q. & He, C. Y., 2010.** A comparative proteomic analysis reveals a new bi-lobe protein required for bi-lobe duplication and cell division in *Trypanosoma brucei*. *PLoS One,* **5** (3): e9660.

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A Golgi-associated bi-lobed structure was previously found to be important for Golgi duplication and cell division in *Trypanosoma brucei*. To further understand its functions, comparative proteomics was performed on extracted flagellar complexes (including the flagellum and flagellum-associated structures such as the basal bodies and the bi-lobe) and purified flagella to identify new bi-lobe proteins. A leucine-rich repeats containing protein, TbLRRP1, was characterized as a new bi-lobe component. The anterior part of the TbLRRP1-labeled bi-lobe is adjacent to the single Golgi apparatus, and the posterior side is tightly associated with the flagellar pocket collar marked by TbBILBO1. Inducible depletion

of TbLRRP1 by RNA interference inhibited duplication of the bi-lobe as well as the adjacent Golgi apparatus and flagellar pocket collar. Formation of a new flagellum attachment zone and subsequent cell division were also inhibited, suggesting a central role of bi-lobe in Golgi, flagellar pocket collar and flagellum attachment zone biogenesis.

15403. **Zimmermann, R., Eyrisch, S., Ahmad, M. & Helms, V., 2010.** Protein translocation across the ER membrane. *Biochimica et Biophysica Acta*. **In press, corrected proof**.

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Protein translocation into the endoplasmic reticulum (ER) is the first and decisive step in the biogenesis of most extracellular and many soluble organelle proteins in eukaryotic cells. It is mechanistically related to protein export from eubacteria and archaea and to the integration of newly synthesized membrane proteins into the ER membrane and the plasma membranes of eubacteria and archaea (with the exception of tail anchored membrane proteins). Typically, protein translocation into the ER involves cleavable amino terminal signal peptides in precursor proteins and sophisticated transport machinery components in the cytosol, the ER membrane, and the ER lumen. Depending on the hydrophobicity and/or overall amino acid content of the precursor protein, transport can occur co- or posttranslationally. The respective mechanism determines the requirements for certain cytosolic transport components. The two mechanisms merge at the level of the ER membrane, specifically, at the heterotrimeric Sec61 complex present in the membrane. The Sec61 complex provides a signal peptide recognition site and forms a polypeptide conducting channel. Apparently, the Sec61 complex is gated by various ligands, such as signal peptides of the transport substrates, ribosomes (in cotranslational transport), and the ER lumenal molecular chaperone, BiP. Binding of BiP to the incoming polypeptide contributes to efficiency and unidirectionality of transport. Recent insights into the structure of the Sec61 complex and the comparison of the transport mechanisms and machineries in the yeast *Saccharomyces cerevisiae*, the human parasite *Trypanosoma brucei*, and mammals have various important mechanistic as well as potential medical implications.

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