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Diminazene diaceturate is used as a trypanocide for cattle in tropical regions. This paper describes a LC-MS (n) method to confirm the presence of diminazene in bovine plasma. Bound diminazene in plasma samples was freed with dilute phosphoric acid, then concentrated on a bonded C (18) SPE cartridge. The LC-MS (n) method utilized electrospray ionization coupled with an ion trap mass spectrometer. Ions observed in MS (2) and MS (3) product ion spectra, as well as those from the MS (1) spectrum, were monitored. The method was validated with plasma samples fortified with diminazene diaceturate (4-100 ng/mL). Diminazene was confirmed in samples fortified with diminazene diaceturate at levels of 6.4 ng/mL or higher.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

[See also **30**: 14138]

14122. **Karlsbakk, E. & Nylund, A., 2006.** Trypanosomes infecting cod *Gadus morhua* L. in the North Atlantic: a resurrection of *Trypanosoma pleuronectidium* Robertson, 1906 and delimitation of *T. murmanense* Nikitin, 1927 (emend.), with a review of other trypanosomes from North Atlantic and Mediterranean teleosts. *Systematic Parasitology*, **65** (3): 175-203.

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Trypanosomes were isolated from Atlantic cod *Gadus morhua* L. collected from several fjords in western Norway. Morphological studies showed that the 12 infections studied represented a single species, identified as *Trypanosoma pleuronectidium* Robertson, 1906 which is resurrected and redescribed. This species is characterised by its body length (57.9 +/- 5.4 microm), nearly central nucleus (NI = 1.05 +/- 0.12) and relatively short post-kinetoplastic (PK) region (3.2 +/- 0.8 microm). *T. pleuronectidium* is transmitted by the leech *Calliobdella nodulifera* (Malm). *T. murmanense* Nikitin, 1927 (emend.) is delimited to a species transmitted by the leech *Johanssonia arctica* (Johansson). This species is separated from *T. pleuronectidium* by its attained body length, more anterior nucleus, presence of cytoplasmic refractive granules, adnuclear vacuoles and by a longer PK region. Partial SSU rDNA sequences of *T. pleuronectidium* and *T. murmanense* from Norway (1980 nt) diverged by 1.9 percent. The nominal North Atlantic and Mediterranean trypanosome species are reviewed, and *T. flesi* Lebailly, 1904, *T. bothi* Lebailly, 1905 and *T. limandae* Brumpt & Lebailly, 1904 are considered synonyms of *T. platessae* Lebailly, 1904. *T. triglae*

senegalensis Ranque, 1973 is not considered conspecific with *T. triglae* Neumann, 1909, and consequently raised to species status as *T. senegalense* Ranque, 1973. Some other likely synonymies are discussed. In addition to *T. pleuronectidium* and *T. murmanense*, the following marine teleost trypanosomes are provisionally listed as valid species pending further study: *T. callionymi* Brumpt & Lebailly, 1904; *T. cotti* Brumpt & Lebailly, 1904; *T. delagei* Brumpt & Lebailly, 1904; *T. dorhni* Yakimov, 1911; *T. gobii* Brumpt & Lebailly, 1904; *T. laternae* Lebailly, 1904; *T. myoxocephali* Fantham, Porter & Richardson, 1942; *T. platessae* Lebailly, 1904; *T. scorpaenae* Neumann, 1909; *T. soleae* Laveran & Mesnil, 1901; *T. triglae* Neumann, 1909; and *T. yakimovi* Yakimov, 1911.

14123. **Lepesheva, G. I., Hargrove, T. Y., Ott, R. D., Nes, W. D. & Waterman, M. R., 2006.** Biodiversity of CYP51 in trypanosomes. *Biochemical Society Transactions*, **34** (6): 1161-1164.

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Sterol 14alpha-demethylases (CYP51) are metabolic cytochromes P450, found in each biological kingdom. They catalyse a single three-step reaction included in all sterol biosynthetic pathways. Plant CYP51s have strict preference towards their physiological substrate O (obtusifoliol), which is C-4-monomethylated. Natural substrates of animal/fungal CYP51 (lanosterol, 24, 25-dihydrolanosterol or 24-methylenelanosterol) are C-4-dimethylated. CYP51 from the pathogenic protozoa TB (*Trypanosoma brucei*) is the first example of O-specific sterol 14alpha-demethylase in non-photosynthetic organisms. Surprisingly, at 83 percent amino acid identity to the TB orthologue, CYP51 from TC (*Trypanosoma cruzi*) clearly prefers C-4-dimethylated sterols. Replacement of animal/fungi-like Ile (105) in the B' helix of TC CYP51 with phenylalanine, the residue found in this position in all plant and other trypanosome CYP51s, dramatically increases the ability of the enzyme to metabolize O, converting it into a more plant-like sterol 14alpha-demethylase. A more than 100-fold increase in binding and turnover is observed for the 24-desmethyl analogue of O [N (norlanosterol)], which is found *in vivo* in procyclic forms of TB and is a good TB CYP51 substrate *in vitro*. We believe that (i) N is a non-conventional CYP51 substrate, preferred in TB and perhaps other *Trypanosomatidae* and (ii) functional similarity of TC CYP51 to animal/fungal orthologues is a result of evolutionary convergence (including F105I mutation), leading to different pathways for sterol production in TC versus TB.

14124. **Maina, N. W., Oberle, M., Otieno, C., Kunz, C., Maeser, P., Ndung'u, J. M. & Brun, R., 2007.** Isolation and propagation of *Trypanosoma brucei gambiense* from sleeping sickness patients in south Sudan. *Transactions of the Royal Societ of Tropical Medicine and Hygiene*, **101** (6): 540-546.

Trypanosomiasis Research Centre (TRC) of KARI, P.O. Box 362, Kikuyu, Kenya, and Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, P.O. Box, CH-4002 Basel, Switzerland.

This study aimed at isolating *Trypanosoma brucei gambiense* from human African trypanosomiasis (HAT) patients from south Sudan. Fifty HAT patients identified during

active screening surveys were recruited, most of whom (49/50) were in second-stage disease. Blood and cerebrospinal fluid samples collected from the patients were cryopreserved using Triladyl ((R)) as the cryomedium. The samples were stored at -150 degrees C in liquid nitrogen vapour in a dry shipper. Eighteen patient stabilates could be propagated in immunosuppressed *Mastomys natalensis* and/or SCID mice. Parasitaemia was highest in SCID mice. Further subpassages in *M. natalensis* increased the virulence of the trypanosomes and all 18 isolates recovered from *M. natalensis* or SCID mice became infective to other immunosuppressed mouse breeds. A comparison of immunosuppressed *M. natalensis* and Swiss White, C57/BL and BALB/c mice demonstrated that all rodent breeds were susceptible after the second subpassage and developed a parasitaemia $>10^6$/ml by Day 5 post infection. The highest parasitaemias were achieved in C57/BL and BALB/c mice. These results indicate that propagation of *T. b. gambiense* isolates after initial isolation in immunosuppressed *M. natalensis* or SCID mice can be done in a range of immunosuppressed rodents.

14125. **Njiru Z. K., Constantine C. C., Gitonga P. K., Thompson, R. C. A. & Reid, S. A., 2007.** Genetic variability of *Trypanosoma evansi* isolates detected by inter-simple sequence repeat anchored-PCR and microsatellite. *Veterinary Parasitology*, **147**(1-2): 51-60.

School of Nursing – Peel Campus, Murdoch University, Carleton Place, 15-17 Mandurah, WA 6150, Australia, Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, P.O. Box 362, Kikuyu 00902, Kenya, Centre for MEGA Epidemiology, School of Population Health, the University of Melbourne, level 2, 723 Swanston Street, Carlton, Vic. 3053, Australia, and School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, WA 6150, Australia. [zknjiru@hotmail.com].

Studies on genetic variability in *Trypanosoma evansi* have been limited by a lack of high-resolution techniques. In this study, we have investigated the use of inter-simple sequence repeats (ISSR) and microsatellites in revealing polymorphism among *T. evansi* isolates. Twelve ISSR primers and five microsatellite loci were used to generate polymorphic bands and alleles, respectively, to investigate the genetic variability among *T. evansi* isolates from Africa and Asia. Seven of the twelve ISSR primers showed variability between isolates with a total of 71 fragments of which 49(69 percent) were polymorphic. Microsatellite analysis revealed a total of 60 alleles. On average the ISSR markers revealed a higher genetic diversity (23 percent) than microsatellites (21.1 percent). The two techniques showed a strong agreement of $r = 0.95$ for Dice and $r = 0.91$ for Jaccard indices in estimating the genetic distances between isolates. The distance UPGMA tree revealed two major clusters of *T. evansi* which correlate with the minicircle classification of subtype A and B. The cophenetic correlation coefficient between Dice and Jaccard based matrices were $r = 0.79$ for microsatellites and $r=0.73$ for ISSR indicating a strong agreement between dendrograms. The results suggest that both ISSR and microsatellites markers are useful in detecting genetic variability within *T. evansi*.

14126. **Shahada, F., Clausen, P-H., Tietjen, U., Chuma, T. & Okamoto, K., 2007.** Absence of correlation between karyotype profiles of *Trypanosoma congolense*

and resistance to isometamidium chloride. *Veterinary Parasitology.* **In press, corrected proof.**

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Konigsweg 67, D-14163 Berlin, Germany, and Laboratory of Veterinary Public Health, Department of Veterinary Medicine, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan. [shahada@ms.kagoshima-u.ac.jp].

Chromosome profiles of 10 *Trypanosoma (T.) congolense* populations with different isometamidium sensitivities were compared using the pulsed field gel electrophoresis technique. The aim was to elucidate whether there was a karyotype pattern specific to eight isometamidium resistant phenotypes. Analysis of the profiles indicated that all populations displayed several discrete bands at the region of small, intermediate and large chromosomes. The highest similarity was observed between two isolates originating from Burkina Faso, indicating that they had the same genetic origin. Other eight strains exhibited different patterns in terms of chromosome size and numbers such that there was no characteristic karyotype pattern that was established specifically to identify resistant populations and discriminate them from the sensitive ones. This study has revealed that isometamidium resistance is not correlated to karyotype profile in *T. congolense.*

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

14127. **Adams, E. R., Malele, I. I, Msangi, A. R. & Gibson, W C., 2006.** Trypanosome identification in wild tsetse populations in Tanzania using generic primers to amplify the ribosomal RNA ITS-1 region. *Acta Tropica*, **100** (1-2): 103-109.

School of Biological Sciences, University of Bristol, Bristol, UK.

Tsetse flies transmit many species of trypanosomes in Africa, some of which are human and livestock pathogens of major medical and socio-economic impact. Identification of trypanosomes is essential to assess the disease risk posed by particular tsetse populations. We have developed a single generic PCR test to replace the multiple species-specific PCR tests used previously to identify the trypanosome species carried by individual tsetse flies. In the generic PCR test, inter-species size variation in the PCR product of the internal transcribed spacer (ITS-1) region of the ribosomal RNA repeat region enables species identification. The test was applied to identify trypanosomes in midgut samples stored on FTA cards from wild-caught flies in two regions of Tanzania. Identifications were verified by sequencing the amplified ITS-1 region and/or species-specific PCR tests. The method facilitated the identification of large numbers of field samples quickly and accurately. Whereas species-specific tests are incapable of recognising previously unknown species, the generic test enabled a new species to be identified by the unique size of the amplified product. Thus, even without access to any isolate of this new species, we could collect data on its distribution, prevalence and co-occurrence with other trypanosomes. The combined molecular and ecological profiles should facilitate the isolation and full biological characterization of this species in the future.

14128. **Al-Salabi, M. I., Wallace, L. J., Luscher, A., Maser, P., Candlish, D., Rodenko, B., Gould, M. K., Jabeen, I., Ajith, S. N. & de Koning, H. P., 2007**. Molecular interactions underlying the unusually high adenosine affinity of a novel *Trypanosoma brucei* nucleoside transporter. *Molecular Pharmacology*, **71** (3): 921-929.

Institute of Biomedical and Life Sciences, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8TA, UK.

Trypanosoma brucei encodes a relatively high number of genes of the equilibrative nucleoside transporter (ENT) family. We report here the cloning and in-depth characterization of one *T. brucei brucei* ENT member, TbNT9/AT-D. This transporter was expressed in *Saccharomyces cerevisiae* and displayed a uniquely high affinity for adenosine (Km = 0.068 +/- 0.013 µM), as well as broader selectivity for other purine nucleosides in the low µM range, but was not inhibited by nucleobases or pyrimidines. This selectivity profile is consistent with the P1 transport activity observed previously in procyclic and long-slender bloodstream *T. brucei*, apart from the 40-fold higher affinity for adenosine than for inosine. We found that, like the previously investigated P1 activity of long/slender bloodstream trypanosomes, the 3'-hydroxy, 5'-hydroxy, N3, and N7 functional groups contribute to transporter binding. In addition, we show that the 6-position amine group of adenosine, but not the inosine 6-keto group, makes a major contribution to binding (DeltaG0 = 12 kJ/mol), explaining the different Km values of the purine nucleosides. We further found that P1 activity in procyclic and long-slender trypanosomes is pharmacologically distinct, and we identified the main gene encoding this activity in procyclic cells as NT10/AT-B. The presence of multiple P1-type nucleoside transport activities in *T. brucei brucei* facilitates the development of nucleoside-based treatments for African trypanosomiasis and would delay the onset of uptake-related drug resistance to such therapy. We show that both TbNT9/AT-D and NT10/AT-B transport a range of potentially therapeutic nucleoside analogues.

14129. **Alsford, S., Kawahara, T., Isamah, C. & Horn, D., 2007**. A sirtuin in the African trypanosome is involved in both DNA repair and telomeric gene silencing but is not required for antigenic variation. *Molecular Microbiology*, **63** (3): 724-736.

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Silent information regulator 2 (Sir2)-related proteins or sirtuins function as NAD^+-dependent deacetylases or ADP ribosylases that target a range of substrates, thereby influencing chromatin structure and a diverse range of other biological functions. Genes encoding three Sir2-related proteins (SIR2rp1-3) have been identified in the parasitic trypanosomatids, early branching protozoa with no previously reported transcriptional silencing machinery. Here we show that, in the mammalian-infective bloodstream-stage of the African trypanosome, *Trypanosoma brucei*, SIR2rp1 localizes to the nucleus while SIR2rp2 and SIR2rp3 are both mitochondrial proteins. The nuclear protein, SIR2rp1, controls DNA repair and repression of RNA polymerase I-mediated expression immediately adjacent to telomeres. Antigenic variation, however, which involves the silencing and Pol I-mediated transcriptional switching of subtelomeric variant surface glycoprotein genes, continues to operate independent of SIR2rp1.

14130. **Babbarwal, V. K., Fleck, M., Ernst, N. L., Schnaufer, A. & Stuart, K., 2007**. An essential role of KREPB4 in RNA editing and structural integrity of the editosome in *Trypanosoma brucei*. *RNA*, **13 (**5): 737-744.

Seattle Biomedical Research Institute, Seattle, Washington 98109, USA.

RNA editing in the sleeping sickness parasite *Trypanosoma brucei* remodels mitochondrial transcripts by the addition and deletion of uridylates as specified by guide RNAs. Editing is catalyzed by at least three distinct approximately 20S multiprotein editosomes, all of which contain KREPB4, a protein with RNase III and Pumilio motifs. RNAi repression of KREPB4 expression in procyclic forms (PFs) strongly inhibited growth and *in vivo* RNA editing, greatly diminished the abundance of 20S editosomes, reduced cellular levels of editosome proteins, and generated approximately 5-10S editosome subcomplexes. Editing TUTase, exoUase, and RNA ligase activities were largely shifted from approximately 20S to approximately 5-10S fractions of cellular lysates. Insertion and deletion endonuclease activities in approximately 20S fractions were strongly reduced upon KREPB4 repression but were not detected in the 5-10S subcomplex fraction. Abundance of MRP1 and RBP16 proteins, which appear to be involved in RNA processing but are not components of the 20S editosome, was unaltered upon KREPB4 repression. These data suggest that KREPB4 is important for the structural integrity of approximately 20S editosomes, editing endonuclease activity, and the viability of PF *T. brucei* cells.

14131. **Barnes, R. L. & McCulloch, R., 2007**. *Trypanosoma brucei* homologous recombination is dependent on substrate length and homology, though displays a differential dependence on mismatch repair as substrate length decreases. *Nucleic Acids Research.* **In press, corrected proof.**

The Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow Biomedical Research Centre, 120 University Place,Glasgow, G12 8TA, UK.

Homologous recombination functions universally in the maintenance of genome stability through the repair of DNA breaks and in ensuring the completion of replication. In some organisms, homologous recombination can perform more specific functions. One example of this is in antigenic variation, a widely conserved mechanism for the evasion of host immunity. *Trypanosoma brucei*, the causative agent of sleeping sickness in Africa, undergoes antigenic variation by periodic changes in its variant surface glycoprotein (VSG) coat. VSG switches involve the activation of VSG genes, from an enormous silent archive, by recombination into specialized expression sites. These reactions involve homologous recombination, though they are characterized by an unusually high rate of switching and by atypical substrate requirements. Here, we have examined the substrate parameters of *T. brucei* homologous recombination. We show, first, that the reaction is strictly dependent on substrate length and that it is impeded by base mismatches, features shared by homologous recombination in all organisms characterized. Second, we identify a pathway of homologous recombination that acts preferentially on short substrates and is impeded to a lesser extent by

base mismatches and the mismatch repair machinery. Finally, we show that mismatches during *T. brucei* recombination may be repaired by short-patch mismatch repair.

14132. **Baron, D. M., Ralston, K. S., Kabututu, Z. P. & Hill, K. L., 2007**. Functional genomics in *Trypanosoma brucei* identifies evolutionarily conserved components of motile flagella. *Journal of Cell Science*, **120** (3): 478-491.

Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095, USA.

Cilia and flagella are highly conserved, complex organelles involved in a variety of important functions. Flagella are required for motility of several human pathogens and ciliary defects lead to a variety of fatal and debilitating human diseases. Many of the major structural components of cilia and flagella are known, but little is known about regulation of flagellar beat. *Trypanosoma brucei*, the causative agent of African sleeping sickness, provides an excellent model for studying flagellar motility. We have used comparative genomics to identify a core group of 50 genes unique to organisms with motile flagella. These genes, referred to as *T. brucei* components of motile flagella (TbCMF) include 30 novel genes, and human homologues of many of the TbCMF genes map to loci associated with human ciliary diseases. To characterize TbCMF protein function we used RNA interference to target 41 TbCMF genes. Sedimentation assays and direct observation demonstrated clear motility defects in a majority of these knockdown mutants. Epitope tagging, fluorescence localization and biochemical fractionation demonstrated flagellar localization for several TbCMF proteins. Finally, ultrastructural analysis identified a family of novel TbCMF proteins that function to maintain connections between outer doublet microtubules, suggesting that they are the first identified components of nexin links. Overall, our results provide insights into the workings of the eukaryotic flagellum, identify several novel human disease gene candidates, reveal unique aspects of the trypanosome flagellum and underscore the value of *T. brucei* as an experimental system for studying flagellar biology.

14133. **Cifuentes-Rojas, C., Pavia, P., Hernandez, A., Osterwisch, D., Puerta, C. & Cruz-Reyes, J., 2007.** Substrate determinants for RNA editing and editing complex interactions at a site for full-round U insertion. *Journal of Biological Chemistry*, **282** (7): 4265-4276.

Department of Biochemistry and Biophysics, Texas A & M University, College Station, Texas 77843, USA.

Multisubunit RNA editing complexes catalyze uridylate insertion/deletion RNA editing directed by complementary guide RNAs (gRNAs). Editing in trypanosome mitochondria is transcript-specific and developmentally controlled, but the molecular mechanisms of substrate specificity remain unknown. Here we used a minimal A6 pre-mRNA/gRNA substrate to define functional determinants for full-round insertion and editing complex interactions at the editing site 2 (ES2). Editing begins with pre-mRNA cleavage within an internal loop flanked by upstream and downstream duplexes with gRNA. We found that substrate recognition around the internal loop is sequence-independent and that completely artificial duplexes spanning a single helical turn are functional. Furthermore, after

our report of cross-linking interactions at the deletion ES1 (35), we show for the first time editing complex contacts at an insertion ES. Our studies using site-specific ribose 2' substitutions defined 2'-hydroxyls within the (a) gRNA loop region and (b) flanking helixes that markedly stimulate both pre-mRNA cleavage and editing complex interactions at ES2. Modification of the downstream helix affected scissile bond specificity. Notably, a single 2'-hydroxyl at ES2 is essential for cleavage but dispensable for editing complex cross-linking. This study provides new insights on substrate recognition during full-round editing, including the relevance of secondary structure and the first functional association of specific (pre-mRNA and gRNA) riboses with both endonuclease cleavage and cross-linking activities of editing complexes at an ES. Importantly, most observed cross-linking interactions are both conserved and relatively stable at ES2 and ES1 in hybrid substrates. However, they were also detected as transient low-stability contacts in a non-edited transcript.

14134. **de Souza Leite, M., Thomaz, R., Fonseca, F. V., Panizzutti, R., Vercesi, A. E. & Meyer-Fernandes, J. R., 2007.** *Trypanosoma brucei brucei*: biochemical characterization of ecto-nucleoside triphosphate diphosphohydrolase activities. *Experimental Parasitology*, **115** (4): 315-323.

Instituto de Bioquimica Medica, CCS, Universidade Federal do Rio de Janeiro, Cidade Universitaria, Rio de Janeiro, RJ, Brazil.

In this work we describe the ability of living cells of *Trypanosoma brucei brucei* to hydrolyze extracellular ATP. In these intact parasites there was a low level of ATP hydrolysis in the absence of any divalent metal (4.72+/-0.51 nmol Pi x 10^{-7} cells x h^{-1}). The ATP hydrolysis was stimulated by $MgCl_2$ and the Mg-dependent ecto-ATPase activity was 27.15+/-2.91 nmol Pi x 10^{-7} cells x h^{-1}. This stimulatory activity was also observed when $MgCl_2$ was replaced by $MnCl_2$. $CaCl_2$ and $ZnCl_2$ were also able to stimulate the ATPase activity, although less than $MgCl_2$. The apparent K (m) for ATP was 0.61 mM. This ecto-ATPase activity was insensitive to inhibitors of other ATPase and phosphatase activities. To confirm that this Mg-dependent ATPase activity is an ecto-ATPase activity, we used an impermeable inhibitor, DIDS (4, 4'-diisothiocyanostylbene 2'-2'-disulphonic acid), as well as suramin, an antagonist of P(2) purinoreceptors and inhibitor of some ecto-ATPases. These two reagents inhibited the Mg^{2+}-dependent ATPase activity in a dose-dependent manner. Living cells sequentially hydrolyzed the ATP molecule generating ADP, AMP and adenosine, and supplementation of the culture medium with ATP was able to sustain the proliferation of *T. brucei brucei* as well as adenosine supplementation. Furthermore, the E-NTPDase activity of *T. brucei brucei* is modulated by the availability of purines in the medium. These results indicate that this surface enzyme may play a role in the salvage of purines from the extracellular medium in *T. brucei brucei*.

14135. **Denninger, V., Figarella, K., Schonfeld, C., Brems, S., Busold, C., Lang, F., Hoheisel, J. & Duszenko, M., 2007.** Troglitazone induces differentiation in *Trypanosoma brucei*. *Experimental Cell Research*, **313** (9): 1805-1819.

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Trypanosoma brucei, a protozoan parasite causing sleeping sickness, is transmitted by the tsetse fly and undergoes a complex lifecycle including several defined stages within the insect vector and its mammalian host. In the latter, differentiation from the long slender to the short stumpy form is induced by a yet unknown factor of trypanosomal origin. Here we describe that some thiazolidinediones are also able to induce differentiation. In higher eukaryotes, thiazolidinediones are involved in metabolism and differentiation processes mainly by binding to intracellular receptors. Our studies focus on the effects of troglitazone on bloodstream form trypanosomes. Differentiation was monitored using mitochondrial markers (membrane potential, succinate dehydrogenase activity, inhibition of oxygen uptake by KCN, amount of cytochrome transcripts), morphological changes (transmission EM and light microscopy), and transformation experiments (loss of the variant surface glycoprotein coat and increase of dihydroliponamide dehydrogenase activity). To further investigate the mechanisms responsible for these changes, microarray analyses were performed, showing an upregulation of expression site associated gene 8 (ESAG8), a potential differentiation regulator.

14136. **Folgueira, C. & Requena, J. M., 2007**. A postgenomic view of the heat shock proteins in kinetoplastids. *FEMS Microbiology Revi*ews. **In press, corrected proof.**

Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain.

The kinetoplastids *Leishmania major, Trypanosoma brucei* and *Trypanosoma cruzi* are causative agents of a diverse spectrum of human diseases: leishmaniasis, sleeping sickness and Chagas' disease, respectively. These protozoa possess digenetic life cycles that involve development in mammalian and insect hosts. It is generally accepted that temperature is a triggering factor of the developmental programme allowing the adaptation of the parasite to the mammalian conditions. The heat shock response is a general homeostatic mechanism that protects cells from the deleterious effects of environmental stresses, such as heat. This response is universal and includes the synthesis of the heat-shock proteins (HSPs). In this review, we summarize the salient features of the different HSP families and describe their main cellular functions. In parallel, we analyse the composition of these families in kinetoplastids according to literature data and our understanding of genome sequence data. The genome sequences of these parasites have been recently completed. The HSP families described here are: HSP110, HSP104, group I chaperonins, HSP90, HSP70, HSP40 and small HSPs. All these families are widely represented in these parasites. In particular, kinetoplastids possess an unprecedented number of members of the HSP70, HSP60 and HSP40 families, suggesting key roles for these HSPs in their biology.

14137. **Galland, N., Demeure, F., Hannaert, V., Verplaetse, E., Vertommen, D., Van der Smissen, P., Courtoy, P. J. & Michels, P. A., 2007.** Characterization of the role of the receptors PEX5 and PEX7 in the import of proteins into glycosomes of *Trypanosoma brucei*. *Biochimica and Biophysica Acta*, **1773** (4): 521-535.

Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology and Laboratory of Biochemistry, Université catholique de Louvain, Brussels, Belgium.

Peroxins 5 and 7 are receptors for protein import into the peroxisomal matrix. We studied the involvement of these peroxins in the biogenesis of glycosomes in the protozoan parasite *Trypanosoma brucei*. Glycosomes are peroxisome-like organelles in which a major part of the glycolytic pathway is sequestered. We here report the characterization of the *T. brucei* homologue of PEX7 and provide several data strongly suggesting that it can bind to PEX5. Depletion of PEX5 or PEX7 by RNA interference had a severe effect on the growth of both the bloodstream-form of the parasite, that relies entirely on glycolysis for its ATP supply, and the procyclic form representative of the parasite living in the tsetse-fly midgut and in which also other metabolic pathways play a prominent role. The role of the two receptors in import of glycosomal matrix proteins with different types of peroxisome/glycosome-targeting signals (PTS) was analyzed by immunofluorescence and subcellular fractionation studies. Knocking down the expression of either receptor gene resulted, in procyclic cells, in the mislocalization of proteins with both a type 1 or 2 targeting motif (PTS1, PTS2) located at the C- and N-termini, respectively, and proteins with a sequence-internal signal (I-PTS) to the cytosol. Electron microscopy confirmed the apparent integrity of glycosomes in these procyclic cells. In bloodstream-form trypanosomes, PEX7 depletion seemed to affect only the subcellular distribution of PTS2-proteins. Western blot analysis suggested that, in both life-cycle stages of the trypanosome, the levels of both receptors are controlled in a coordinated fashion, by a mechanism that remains to be determined. The observation that both PEX5 and PEX7 are essential for the viability of the parasite indicates that the respective branches of the glycosome-import pathway in which each receptor acts might be interesting drug targets.

14138. **Gibson, W., 2007**. Resolution of the species problem in African trypanosomes. *International Journal of Parasitology*, **37** (8-9): 829-838.

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There is a general assumption that eukaryote species are demarcated by morphological or genetic discontinuities. This stems from the idea that species are defined by the ability of individuals to mate and produce viable progeny. At the microscopic level, where organisms often proliferate more by asexual than sexual reproduction, this tidy classification system breaks down and species definition becomes messy and problematic. The dearth of morphological characters to distinguish microbial species has led to the widespread application of molecular methods for identification. As well as providing molecular markers for species identification, gene sequencing has generated the data for accurate estimation of relatedness between different populations of microbes. This has led to recognition of conflicts between current taxonomic designations and phylogenetic placement. In the case of microbial pathogens, the extent to which taxonomy has been driven by utilitarian rather than biological considerations has been made explicit by molecular phylogenetic analysis. These issues are discussed with reference to the taxonomy of the African trypanosomes, where pathogenicity, host range and distribution have been influential in the designation of species and subspecies.

Effectively, the taxonomic units recognised are those that are meaningful in terms of human or animal disease. The underlying genetic differences separating the currently recognised trypanosome taxa are not consistent, ranging from genome-wide divergence to presence/absence of a single gene. Nevertheless, if even a minor genetic difference reflects adaptation to a particular parasitic niche, for example, in *Trypanosoma brucei rhodesiense*, the presence of a single gene conferring the ability to infect humans, then it can prove useful as an identification tag for the taxon occupying that niche. Thus, the species problem can be resolved by bringing together considerations of utility, genetic difference and adaptation.

14139. **Glover, L., Alsford, S., Beattie, C. & Horn, D., 2007.** Deletion of a trypanosome telomere leads to loss of silencing and progressive loss of terminal DNA in the absence of cell cycle arrest. *Nucleic Acids Research*, **35** (3): 872-880.

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Eukaryotic chromosomes are capped with telomeres which allow complete chromosome replication and prevent the ends from being recognized by the repair machinery. The African trypanosome, *Trypanosoma brucei*, is a protozoan parasite where antigenic variation requires reversible silencing of a repository of telomere-adjacent variant surface glycoprotein (VSG) genes. We have investigated the role of the telomere adjacent to a repressed VSG. In cells lacking telomerase, the rate of telomere-repeat loss appeared to be inversely proportional to telomere length. We therefore constructed strains in which a single telomere could be immediately removed by conditional I-SceI meganuclease cleavage. Following telomere deletion, cells maintain and segregate the damaged chromosome without repairing it. These cells continue to proliferate at the normal rate but progressively lose terminal DNA at the broken end. Although sirtuin-dependent repression is lost along with the telomere, VSG-silencing is preserved. The results provide direct evidence for telomere-dependent repression but suggest a telomere-independent mode of VSG-silencing. They also indicate the absence of a telomere-loss checkpoint in *T. brucei*.

14140. **Goulah, C. C. & Read, L. K., 2007.** Differential effects of arginine methylation on RBP16 mRNA binding, guide RNA (gRNA) binding, and gRNA-containing ribonucleoprotein complex (gRNP) formation. *Journal of Biological Chemistry*, **282** (10): 7181-7190.

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Mitochondrial gene expression in *Trypanosoma brucei* involves the coordination of multiple events including polycistronic transcript cleavage, polyadenylation, RNA stability, and RNA editing. Arg methylation of RNA binding proteins has the potential to influence many of these processes via regulation of protein-protein and protein-RNA interactions. Here we demonstrate that Arg methylation differentially regulates the RNA binding capacity and macromolecular interactions of the mitochondrial gene regulatory protein, RBP16. We show that, in *T. brucei* mitochondria, RBP16 forms two major stable complexes: a 5 S multiprotein

complex and an 11 S complex consisting of the 5 S complex associated with guide RNA (gRNA). Expression of a non-methylatable RBP16 mutant protein demonstrates that Arg methylation of RBP16 is required to maintain the protein-protein interactions necessary for assembly and/or stability of both complexes. Down-regulation of the major trypanosome type 1 protein arginine methyltransferase, *Tb*PRMT1, disrupts formation of both the 5 and 11 S complexes, indicating that *Tb*PRMT1-catalyzed methylation of RBP16 Arg-78 and Arg-85 is critical for complex formation. We also show that Arg methylation decreases the capacity of RBP16 to associate with gRNA. This is not a general effect on RBP16 RNA binding, however, since methylation conversely increases the association of the protein with mRNA. Thus, *Tb*PRMT1-catalyzed Arg methylation has distinct effects on RBP16 gRNA and mRNA association and gRNA-containing ribonucleoprotein complex (gRNP) formation.

14141. **Gourguechon S., Savich, J. M. & Wang, C. C., 2007.** The multiple roles of cyclin E1 in controlling cell cycle progression and cellular morphology of *Trypanosoma brucei*. *Journal of Molecular Biology*, **368** (4): 939-950.

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Regulation of eukaryotic cell cycle progression requires sequential activation and inactivation of cyclin-dependent kinases. Previous RNA interference (RNAi) experiments in *Trypanosoma brucei* indicated that cyclin E1, cdc2-related kinase (CRK)1 and CRK2 are involved in regulating G1/S transition, whereas cyclin B2 and CRK3 play a pivotal role in controlling the G2/M checkpoint. To search for potential interactions between the other cyclins and CRKs that may not have been revealed by the RNAi assays, we used the yeast two-hybrid system and an *in vitro* glutathione-S-transferase pulldown assay and observed interactions between cyclin E1 and CRK1, CRK2 and CRK3. Cyclins E1–E4 are homologues of yeast Pho80 cyclin. But yeast complementation assays indicated that none of them possesses a Pho80-like function. Analysis of cyclin E1 + CRK1 and cyclin E1 + CRK2 double knockdowns in the procyclic form of *T. brucei* indicated that the cells were arrested more extensively in the G1 phase beyond the cumulative effect of individual knockdowns. But BrdU incorporation was impaired significantly only in cyclin E1 + CRK1-depleted cells, whereas a higher percentage of cyclin E1 + CRK2 knockdown cells assumed a grossly elongated posterior end morphology. A double knockdown of cyclin E1 and CRK3 arrested cells in G2/M much more efficiently than if only CRK3 was depleted. Taken together, these data suggest multiple functions of cyclin E1: it forms a complex with CRK1 in promoting G1/S phase transition; it forms a complex with CRK2 in controlling the posterior morphogenesis during G1/S transition; and it forms a complex with CRK3 in promoting passage across the G2/M checkpoint in the trypanosome.

14142. **Hammarton, T. C., 2007.** Cell cycle regulation in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **153** (1): 1-8.

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Cell division is regulated by intricate and interconnected signal transduction pathways that precisely coordinate, in time and space, the complex series of events involved in replicating and segregating the component parts of the cell. In *Trypanosoma brucei*, considerable progress has been made over recent years in identifying molecular regulators of the cell cycle and elucidating their functions, although many regulators undoubtedly remain to be identified, and there is still a long way to go with respect to determining signal transduction pathways. However, it is clear that cell cycle regulation in *T. brucei* is unusual in many respects. Analyses of trypanosome orthologues of conserved eukaryotic cell cycle regulators have demonstrated divergence of their function in the parasite, and a number of other key regulators are missing from *T. brucei.* Cell cycle regulation differs in different parasite life cycle stages, and *T. brucei* appears to use different checkpoint control strategies compared to model eukaryotes. It is therefore probable that *T. brucei* has evolved novel pathways to control its cell cycle.

14143. **Hee Lee, S., Stephens, J. L. & Englund, P. T., 2007.** A fatty-acid synthesis mechanism specialized for parasitism. *Nature Reviews, Microbiology*, **5** (4): 287-297.

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Most cells use either a type I or type II synthase to make fatty acids. *Trypanosoma brucei*, the sleeping sickness parasite, provides the first example of a third mechanism for this process. Trypanosomes use microsomal elongases to synthesize fatty acids *de novo*, whereas other cells use elongases to make long-chain fatty acids even longer. The modular nature of the pathway allows synthesis of different fatty-acid end products, which have important roles in trypanosome biology. Indeed, this newly discovered mechanism seems ideally suited for the parasitic lifestyle.

14144. **Kang, X., Gao, G., Rogers, K., Falick, A. M., Zhou, S. & Simpson, L., 2006**. Reconstitution of full-round uridine-deletion RNA editing with three recombinant proteins. *Proceedings of the National Academy of Sciences USA*, **103** (38): 13944-13949.

Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095, USA.

Uridine (U)-insertion/deletion RNA editing in trypanosome mitochondria involves an initial cleavage of the preedited mRNA at specific sites determined by the annealing of partially complementary guide RNAs. An involvement of two RNase III-containing core editing complex (L-complex) proteins, MP90 (KREPB1) and MP61 (KREPB3) in, respectively, U-deletion and U-insertion editing, has been suggested, but these putative enzymes have not been characterized or expressed in active form. Recombinant MP90 proteins from *Trypanosoma brucei* and *Leishmania major* were expressed in insect cells and cytosol of *Leishmania tarentolae*, respectively. These proteins were active in specifically cleaving a model U-deletion site and not a U-insertion site. Deletion or mutation of the RNase III motif abolished this activity. Full-round guide RNA (gRNA)-mediated *in vitro* U-

deletion editing was reconstituted by a mixture of recombinant MP90 and recombinant RNA editing exonuclease I from *L. major*, and recombinant RNA editing RNA ligase 1 from *L. tarentolae*. MP90 is designated REN1, for RNA-editing nuclease 1.

14145. **Law, J. A., O'Hearn, S. & Sollner-Webb, B., 2007**. In *Trypanosoma brucei* RNA editing, *Tb*MP18 (band VII) is critical for editosome integrity and for both insertional and deletional cleavages. *Molecular and Cellular Biology*, **27** (2): 777-787.

Department of Biological Chemistry, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA.

In trypanosome RNA editing, uridylate (U) residues are inserted and deleted at numerous sites within mitochondrial pre-mRNAs by an approximately 20S protein complex that catalyzes cycles of cleavage, U addition/U removal, and ligation. We used RNA interference to deplete *Tb*MP18 (band VII), the last unexamined major protein of our purified editing complex, showing it is essential. *Tb*MP18 is critical for the U-deletional and U-insertional cleavages and for integrity of the approximately 20S editing complex, whose other major components, *Tb*MP99, *Tb*MP81, *Tb*MP63, *Tb*MP52, *Tb*MP48, *Tb*MP42 (bands I through VI), and *Tb*MP57, instead sediment as approximately 10S associations. Additionally, *Tb*MP18 augments editing substrate recognition by the *Tb*MP57 terminal U transferase, possibly aiding the recognition component, *Tb*MP81. The other editing activities and their coordination in precleaved editing remain active in the absence of *Tb*MP18. These data are reminiscent of the data on editing subcomplexes reported by A. Schnaufer *et al.* (*Mol. Cell* **12**: 307-319, 2003) and suggest that these subcomplexes are held together in the approximately 20S complex by *Tb*MP18, as was proposed previously. Our data additionally imply that the proteins are less long-lived in these subcomplexes than they are when held in the complete editing complex. The editing endonucleolytic cleavages being lost when the editing complex becomes fragmented, as upon *Tb*MP18 depletion, should be advantageous to the trypanosome, minimizing broken mRNAs.

14146. **Laxman, S., Riechers, A., Sadilek, M., Schwede, F. & Beavo, J. A., 2006.** Hydrolysis products of cAMP analogues cause transformation of *Trypanosoma brucei* from slender to stumpy-like forms. *Proceedings of the National Academy of Sciences USA*, **103** (50): 19194-19199.

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African sleeping sickness is a disease caused by *Trypanosoma brucei*. *T. brucei* proliferates rapidly in the mammalian bloodstream as long, slender forms, but at higher population densities they transform into nondividing, short, stumpy forms. This is thought to be a mechanism adopted by *T. brucei* to establish a stable host-parasite relationship and to allow a transition into the insect stage of its life cycle. Earlier studies have suggested a role for cAMP in mediating this transformation. In this study, using membrane-permeable nucleotide analogues, we show that it is not the cAMP analogues themselves but rather the hydrolyzed products of membrane-permeable cAMP analogues that prevent proliferation of

T. brucei. The metabolic products are more potent than the cAMP analogues, and hydrolysis-resistant cAMP analogues are not antiproliferative. We further show that the antiproliferative effect of these membrane-permeable adenosine analogues is caused by transformation into forms resembling short, stumpy bloodstream forms. These data suggest that the slender-to-stumpy transformation of *T. brucei* may not be mediated directly by cAMP and also raise the possibility of using such adenosine analogues as antitrypanosomal drugs.

14147. **Lecordier, L., Devaux, S., Uzureau, P., Dierick, J. F., Walgraffe, D., Poelvoorde, P., Pays, E. & Vanhamme, L., 2007.** Characterization of a TFIIH homologue from *Trypanosoma brucei*. *Molecular Microbiology*, **64** (5): 1164-1181.

Laboratory of Molecular Parasitology, Institute of Molecular Biology and Medicine, Université Libre de Bruxelles, 12, rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium.

Trypanosomes are protozoans showing unique transcription characteristics. We describe in *Trypanosoma brucei* a complex homologous to TFIIH, a multisubunit transcription factor involved in the control of transcription by RNA Pol I and RNA Pol II, but also in DNA repair and cell cycle control. Bioinformatics analyses allowed the detection of five genes encoding four putative core TFIIH subunits (*Tb*XPD, *Tb*XPB, *Tb*p44, *Tb*p52), including a novel XPB variant, *Tb*XPBz. In all cases sequences known to be important for TFIIH functions were conserved. We performed a molecular analysis of this core complex, focusing on the two subunits endowed with a known enzymatic (helicase) activity, XPD and XPB. The involvement of these *T. brucei* proteins in a *bona fide* TFIIH core complex was supported by (i) colocalization by immunofluorescence in the nucleus, (ii) direct physical interaction of *Tb*XPD and its interacting regulatory subunit *Tb*p44 as determined by double-hybrid assay and tandem affinity purification of the core TFIIH, (iii) involvement of the core proteins in a high molecular weight complex and (iv) occurrence of transcription, cell cycle and DNA repair phenotypes upon either RNAi knock-down or overexpression of essential subunits.

14148. **Marcello, L. & Barry, J. D., 2007.** From silent genes to noisy populations -dialogue between the genotype and phenotypes of antigenic variation. *Journal of Eukaryotic Microbiology*, **54** (1): 14-17.

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African trypanosomes evade humoral immunity through antigenic variation whereby they switch expression of the variant surface glycoprotein (VSG) gene encoding their glycoprotein surface coat. Switching proceeds by duplication from an archive of silent VSG genes into a transcriptionally active locus, and precedent suggests silent genes can contribute, combinatorially to formation of expressed, functional genes through segmental gene conversion. The genome project has revealed that most of the silent archive consists of hundreds of VSG genes in subtelomeric tandem arrays, and that most of these are not functional genes. The aim of this review is to explore links between the uncovered trypanosome genotype and the phenotype of antigenic variation, stretching from the broad

phenotype-transmission in the field and the overcoming of herd immunity to events within single infections. Highlighting in particular the possible impact of phenotype selection on the evolution of the VSG archive and the mechanisms for its expression leads to a theoretical framework to further our understanding of this complex immune evasion strategy.

14149. **Martinez-Oyanedel, J., McNae, I. W., Nowicki, M. W., Keillor, J. W., Michels, P. A., Fothergill-Gilmore, L. A. & Walkinshaw, M. D., 2007**. The first crystal structure of phosphofructokinase from a eukaryote: *Trypanosoma brucei*. *Journal of Molecular Biology*, **366** (4): 1185-1198.

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The crystal structure of the ATP-dependent phosphofructokinase (PFK) from *Trypanosoma brucei* provides the first detailed description of a eukaryotic PFK, and enables comparisons to be made with the crystal structures of bacterial ATP-dependent and PPi-dependent PFKs. The structure reveals that two insertions (the 17-20 and 329-348 loops) that are characteristic of trypanosomatid PFKs, but absent from bacterial and mammalian ATP-dependent PFKs, are located within and adjacent to the active site, and are in positions to play important roles in the enzyme's mechanism. The 90 residue N-terminal extension forms a novel domain that includes an "embracing arm" across the subunit boundary to the symmetry-related subunit in the tetrameric enzyme. Comparisons with the PPi-dependent PFK from *Borrelia burgdorferi* show that several features thought to be characteristic of PPi-dependent PFKs are present in the trypanosome ATP-dependent PFK. These two enzymes are generally more similar to each other than to the bacterial or mammalian ATP-dependent PFKs. However, there are critical differences at the active site of PPi-dependent PFKs that are sufficient to prevent the binding of ATP. This crystal structure of a eukaryotic PFK has enabled us to propose a detailed model of human muscle PFK that shows active site and other differences that offer opportunities for structure-based drug discovery for the treatment of sleeping sickness and other diseases caused by the trypanosomatid family of protozoan parasites.

14150. **Morrison L. J., McCormack, G., Sweeney L., Likeufack, A. C., Truc, P., Turner, C. M., Tait, A. & Macleod, A., 2007.** Use of multiple displacement amplification to increase the detection and genotyping of *Trypanosoma* species samples immobilized on FTA filters. *American Journal of Tropical Medicine and Hygiene,* **76** (6): 1132-1137.

Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK.; Institut de Recherche pour le Développement, UR 177 Trypanosomoses Africaines, Montpellier, France; Institut de Recherche pour le Développement, UR 177 Trypanosomoses Africaines, Luanda, Angola; and Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

Whole genome amplification methods are a recently developed tool for amplifying DNA from limited template. We report its application in trypanosome infections, characterized by low parasitaemias. Multiple displacement amplification (MDA) amplifies

DNA with a simple *in vitro* step and was evaluated on mouse blood samples on FTA filter cards with known numbers of *Trypanosoma brucei* parasites. The data showed a 20-fold increase in the number of PCRs possible per sample, using primers diagnostic for the multicopy ribosomal ITS region or 177-bp repeats, and a 20-fold increase in sensitivity over nested PCR against a single-copy microsatellite. Using MDA for microsatellite genotyping caused allele dropout at low DNA concentrations, which was overcome by pooling multiple MDA reactions. The validity of using MDA was established with samples from Human African Trypanosomiasis patients. The use of MDA allows maximal use of finite DNA samples and may prove a valuable tool in studies where multiple reactions are necessary, such as population genetic analyses.

14151. **Navarro, M., Peñate, X. & Landeira, D., 2007.** Nuclear architecture underlying gene expression in *Trypanosoma brucei*. *Trends in Microbiology*, **15** (6): 263-270.

Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas (Spanish National Research Council), Avda. del Conocimiento s/n, 18100 Granada, Spain. [miguel.navarro@ipb.csic.es].

The influence of nuclear architecture on the regulation of developmental gene expression has recently become evident in many organisms ranging from yeast to humans. During interphase, chromosomes and nuclear structures are in constant motion; therefore, correct temporal association is needed to meet the requirements of gene expression. *Trypanosoma brucei* is an excellent model system in which to analyze nuclear spatial implications in the regulation of gene expression because the two main surface-protein genes (procyclin and VSG) are transcribed by the highly compartmentalized RNA polymerase I and undergo distinct transcriptional activation or downregulation during developmental differentiation. Furthermore, the infective bloodstream form of the parasite undergoes antigenic variation, displaying sequentially different types of VSG by allelic exclusion. Here, we discuss recent advances in understanding the role of chromosomal nuclear positioning in the regulation of gene expression in *T. brucei.*

14152. **Nowicki, C. & Cazzulo, J. J., 2007.** Aromatic amino acid catabolism in trypanosomatids. *Comparative Biochemistry and Physiology*. **In press, corrected proof.**

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Trypanosomatids cause important human diseases, like sleeping sickness, Chagas disease, and the leishmaniases. Unlike in the mammalian host, the metabolism of aromatic amino acids is a very simple pathway in these parasites. *Trypanosoma brucei* and *Trypanosoma cruzi* transaminate the three aromatic amino acids, the resulting 2-oxo acids being reduced to the corresponding lactate derivatives and excreted. In *T. cruzi*, two enzymes are involved in this process: a tyrosine aminotransferase (TAT), which despite a high sequence similarity with the mammalian enzyme, has a different substrate specificity; and an aromatic L-2-hydroxyacid dehydrogenase (AHADH), which belongs to the subfamily of the cytosolic malate dehydrogenases (MDHs), yet has no MDH activity. In *T. cruzi* AHADH the

substitution of Ala102 for Arg enables AHADH to reduce oxaloacetate. In the members of the 2-hydroxyacid dehydrogenases family, the residue at this position is known to be responsible for substrate specificity. *T. cruzi* does not possess a cytosolic MDH but contains a mitochondrial and a glycosomal MDH; by contrast *T. brucei* and *Leishmania* spp. possess a cytosolic MDH in addition to glycosomal and mitochondrial isozymes. Although *Leishmania mexicana* also transaminates aromatic amino acids through a broad specificity aminotransferase, the latter presents low sequence similarity with TATs, and this parasite does not seem to have an enzyme equivalent to *T. cruzi* AHADH. Therefore, these closely related primitive eukaryotes have developed aromatic amino acid catabolism systems using different enzymes and probably for different metabolic purposes.

14153. **Oberholzer, M., Marti, G., Baresic, M., Kunz, S., Hemphill, A. & Seebeck, T., 2007.** The *Trypanosoma brucei* cAMP phosphodiesterases *Tbr*PDEB1 and *Tbr*PDEB2: flagellar enzymes that are essential for parasite virulence. *FASEB Journal*, **21** (3): 720-731.

Institute of Cell Biology, University of Bern, Switzerland.

Cyclic nucleotide specific phosphodiesterases (PDEs) are pivotal regulators of cellular signalling. They are also important drug targets. Besides catalytic activity and substrate specificity, their subcellular localization and interaction with other cell components are also functionally important. In contrast to the mammalian PDEs, the significance of PDEs in protozoal pathogens remains mostly unknown. The genome of *Trypanosoma brucei,* the causative agent of human sleeping sickness, codes for five different PDEs. Two of these, *Tbr*PDEB1 and *Tbr*PDEB2, are closely similar, cAMP-specific PDEs containing two GAF-domains in their N-terminal regions. Despite their similarity, these two PDEs exhibit different subcellular localizations. *Tbr*PDEB1 is located in the flagellum, whereas *Tbr*PDEB2 is distributed between flagellum and cytoplasm. RNAi against the two mRNAs revealed that the two enzymes can complement each other but that a simultaneous ablation of both leads to cell death in bloodstream form trypanosomes. RNAi against *Tbr*PDEB1 and *Tbr*PDEB2 also functions *in vivo* where it completely prevents infection and eliminates ongoing infections. Our data demonstrate that *Tbr*PDEB1 and *Tbr*PDEB2 are essential for virulence, making them valuable potential targets for new PDE-inhibitor based trypanocidal drugs. Furthermore, they are compatible with the notion that the flagellum of *T. brucei* is an important site of cAMP signalling.

14154. **Ochsenreiter, T. & Hajduk, S. L., 2006**. Alternative editing of cytochrome c oxidase III mRNA in trypanosome mitochondria generates protein diversity. *EMBO Reports*, **7** (11): 1128-1133.

Program in Global Infectious Diseases, Josephine Bay Paul Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, Massachusetts 02543, USA.

Trypanosomes use RNA editing to produce most functional mitochondrial messenger RNA. Precise insertion and deletion of hundreds of uridines is necessary to make full-length cytochrome c oxidase III (COXIII) mRNA. We show that COXIII mRNA can be alternatively edited by a mechanism using an alternative guide RNA to make a stable mRNA.

This alternatively edited mRNA is translated to produce a unique protein that fractionates with mitochondrial membranes and colocalizes with mitochondrial proteins *in situ*. Alternative RNA editing represents a previously unknown mechanism generating protein diversity and, as such, represents an important function for RNA editing.

14155. **Ott, R., Chibale, K., Anderson, S., Chipeleme, A., Chaudhuri, M., Guerrah, A., Colowick, N. & Hill, G. C., 2006.** Novel inhibitors of the trypanosome alternative oxidase inhibit *Trypanosoma brucei brucei* growth and respiration. *Acta Tropica*, **100** (3): 172-184.

Vanderbilt University School of Medicine, Department of Microbiology and Immunology, Nashville, TN 37232, USA.

African trypanosomiasis is a deadly disease for which few chemotherapeutic options are available. The causative agents, *Trypanosoma brucei rhodesiense* and *T. b. gambiense*, utilize a non-cytochrome, alternative oxidase (AOX) for their cellular respiration. The absence of this enzyme in mammalian cells makes it a logical target for therapeutic agents. We designed three novel compounds, ACB41, ACD15, and ACD16, and investigated their effects on trypanosome alternative oxidase (TAO) enzymatic activity, parasite respiration, and parasite growth *in vitro*. All three compounds contain a 2-hydroxybenzoic acid moiety, analogous to that present in SHAM, and a prenyl side chain similar to that found in ubiquinol. ACD15 and ACD16 are further differentiated by the presence of a solubility-enhancing carbohydrate moiety. Kinetic studies with purified TAO show that all three compounds competitively inhibit TAO, and two compounds, ACB41 and ACD15, have inhibition constants five- and three-fold more potent than SHAM, respectively. All three compounds inhibited the respiration and growth of continuously cultured *T. b. brucei* bloodstream cells in a dose-dependent manner. None of the compounds interfered with respiration of rat liver mitochondria, nor did they inhibit the growth of a continuously cultured mammalian cell line. Collectively, the results suggest we have identified a new class of compounds that are inhibitors of TAO, have trypanocidal properties *in vitro*, and warrant further investigation *in vivo*.

14156. **Persson, L., 2007**. Ornithine decarboxylase and S-adenosylmethionine decarboxylase in trypanosomatids. *Biochemical Society Transactions*, **35 (**2): 314-317.

Department of Experimental Medical Science, Lund University, BMC F:13, S-221 84 Lund, Sweden. [lo.persson@med.lu.se].

The production of polyamines has been shown to be an effective target for a drug against the West African form of sleeping sickness caused by *Trypanosoma brucei gambiense*. *T. brucei* belongs to the group of protozoan parasites classed as trypanosomatids. Parasitic species of this group are the causative agents of various tropical diseases besides African sleeping sickness, e.g. Chagas' disease (*Trypanosoma cruzi*), cutaneous (*Leishmania* spp.) and visceral (*Leishmania donovani*) leishmaniasis. The metabolism of polyamines in the parasites is a potential target for the development of new drugs for treatment of these diseases. The key steps in polyamine synthesis are catalysed by ODC (ornithine decarboxylase) and AdoMetDC (S-adenosylmethionine decarboxylase). In the present paper,

some of the available information on ODC and AdoMetDC in trypanosomatids will be described and discussed.

14157. **Richmond, G. S. & Smith, T. K., 2007**. A novel phospholipase from *Trypanosoma brucei*. *Molecular Microbiology*, **63** (4): 1078-1095.

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Phospholipase A (1) activities have been detected in most cells where they have been sought and yet their characterization lags far behind that of the phospholipases A(2), C and D. The study presented here details the first cloning and characterization of a cytosolic PLA (1) that exhibits preference for phosphatidylcholine (GPCho) substrates. *Trypanosoma brucei* phospholipase A (1) (*Tb*PLA(1)) is unique from previously identified eukaryotic PLA(1) because it is evolutionarily related to bacterial secreted PLA(1). A *T. brucei* ancestor most likely acquired the PLA (1) from a horizontal gene transfer of a PLA (1) from *Sodalis glossinidius*, a bacterial endosymbiont of tsetse flies. Nano-electrospray ionization tandem mass spectrometry analysis of *Tb*PLA (1) mutants established that the enzyme functions *in vivo* to synthesize lysoGPCho metabolites containing long-chain mostly polyunsaturated and highly unsaturated fatty acids. Analysis of purified mutated recombinant forms of *Tb*PLA (1) revealed that this enzyme is a serine hydrolase whose catalytic mechanism involves a triad consisting of the amino acid residues Ser-131, His-234 and Asp-183. The *Tb*PLA (1) homozygous null mutants generated here constitute the only PLA (1) double knockouts from any organism.

14158. **Schlecker, T., Comini, M. A., Melchers, J., Ruppert, T. & Krauth-Siegel, R. L., 2007.** Catalytic mechanism of the glutathione peroxidase-type tryparedoxin peroxidase of *Trypanosoma brucei*. *Biochemical Journal*. **In press, corrected proof**.

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Trypanosoma brucei, the causative agent of African sleeping sickness, encodes three nearly identical genes for cysteine-homologues of the selenocysteine-containing glutathione peroxidases. The enzymes - which are essential for the parasites - lack glutathione peroxidase activity but catalyze the trypanothione/tryparedoxin-dependent reduction of hydroperoxides. Cys-47, Gln-82, and Trp-137 correspond to the SeCys, Gln, and Trp catalytic triad of the mammalian selenoenzymes. Site directed mutagenesis revealed that Cys-47 and Gln-82 are essential. A glycine mutant of Trp-137 had 13 percent of wild-type activity which suggests that the aromatic residue may play a structural role but is not directly involved in catalysis. Cys-95, conserved in related yeast and plant proteins but not in the mammalian selenoenzymes, proved to be essential as well. In contrast, replacement of the highly conserved Cys-76 by a serine resulted in a fully active enzyme species and its role remains unknown. Thr-50, proposed to stabilize the thiolate anion at Cys-47, is also not essential for catalysis. Treatment of the C76S/C95S but not of the C47S/C76S double mutant with H_2O_2 induced formation of a sulphinic acid and covalent homodimers in accordance with Cys-47 being the peroxidative active site thiol. In the wild-type peroxidase, these oxidations are

prevented by formation of an intramolecular disulfide bridge between Cys-47 and Cys-95. As shown by mass spectrometry, regeneration of the reduced enzyme by tryparedoxin involves a transient mixed disulfide between Cys-95 of the peroxidase and Cys-40 of tryparedoxin. The catalytic mechanism of the tryparedoxin peroxidase resembles that of atypical 2-Cys-peroxiredoxins but is distinct from that of the selenoenzymes.

14159. **Scory, S., Stierhof, Y. D., Caffrey, C. R. & Steverding, D., 2007.** The cysteine proteinase inhibitor Z-Phe-Ala-CHN2 alters cell morphology and cell division activity of *Trypanosoma brucei* bloodstream forms *in vivo*. *Kinetoplastid Bioloy and Disease*, 6: 2.

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Current chemotherapy of human African trypanosomiasis or sleeping sickness relies on drugs developed decades ago, some of which show toxic side effects. One promising line of research towards the development of novel anti-trypanosomal drugs are small-molecule inhibitors of *Trypanosoma brucei* cysteine proteinases. In this study, we demonstrate that treatment of *T. brucei*-infected mice with the inhibitor, carbobenzoxy-phenylalanyl-alanine-diazomethyl ketone (Z-Phe-Ala-CHN_2), alters parasite morphology and inhibits cell division. Following daily intra-peritoneal administration of 250 mg kg^{-1} of Z-Phe-Ala-CHN_2 on days three and four post infection (p.i.), stumpy-like forms with enlarged lysosomes were evident by day five p.i. In addition, trypanosomes exposed to the inhibitor had a 65 percent greater protein content than those from control mice. Also, in contrast to the normal 16 percent of parasites containing two kinetoplasts - a hallmark of active mitosis, only 4 percent of trypanosomes exposed to the inhibitor were actively dividing, indicating cell cycle-arrest. We suggest that inhibition of endogenous cysteine proteinases by Z-Phe-Ala-CHN_2 depletes the parasite of essential nutrients necessary for DNA synthesis, which in turn, prevents progression of the cell cycle. This arrest then triggers differentiation of the long slender into short-stumpy forms.

14160. **Stephens, J. L., Lee, S. H., Paul, K. S. & Englund, P. T., 2007.** Mitochondrial fatty acid synthesis in *Trypanosoma brucei*. *Journal of Biological Chemistry*, **282** (7): 4427-4436.

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Whereas other organisms utilize type I or type II synthases to make fatty acids, trypanosomatid parasites such as *Trypanosoma brucei* are unique in their use of a microsomal elongase pathway (ELO) for *de novo* fatty acid synthesis (FAS). Because of the unusual lipid metabolism of the trypanosome, it was important to study a second FAS pathway predicted by the genome to be a type II synthase. We localized this pathway to the mitochondrion, and RNA interference (RNAi) or genomic deletion of acyl carrier protein (ACP) and beta-ketoacyl-ACP synthase indicated that this pathway is likely essential for bloodstream and procyclic life cycle stages of the parasite. *In vitro* assays show that the largest major fatty

acid product of the pathway is C_{16}, whereas the ELO pathway, utilizing ELOs 1, 2, and 3, synthesizes up to C_{18}. To demonstrate mitochondrial FAS *in vivo*, we radioabelled fatty acids in cultured procyclic parasites with ^{14}C pyruvate or ^{14}C threonine, either of which is catabolized to ^{14}C acetyl-CoA in the mitochondrion. Although some of the ^{14}C acetyl-CoA may be utilized by the ELO pathway, a striking reduction in radiolabelled fatty acids following ACP RNAi confirmed that it is also consumed by mitochondrial FAS. ACP depletion by RNAi or gene knockout also reduces lipoic acid levels and drastically decreases protein lipoylation. Thus, octanoate (C_8), the precursor for lipoic acid synthesis, must also be a product of mitochondrial FAS. Trypanosomes employ two FAS systems: the unconventional ELO pathway that synthesizes bulk fatty acids and a mitochondrial pathway that synthesizes specialized fatty acids that are likely utilized intramitochondrially.

14161. **Urwyler, S., Studer, E., Renggli, C. K. & Roditi, I., 2007.** A family of stage-specific alanine-rich proteins on the surface of epimastigote forms of *Trypanosoma brucei*. *Molecular Microbiology*, **63** (1): 218-228.

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A “two coat” model of the life cycle of *Trypanosoma brucei* has prevailed for more than 15 years. Metacyclic forms transmitted by infected tsetse flies and mammalian bloodstream forms are covered by variant surface glycoproteins. All other life cycle stages were believed to have a procyclin coat, until it was shown recently that epimastigote forms in tsetse salivary glands express procyclin mRNAs without translating them. As epimastigote forms cannot be cultured, a procedure was devised to compare the transcriptomes of parasites in different fly tissues. Transcripts encoding a family of glycosylphosphatidyl inositol-anchored proteins, BARPs (previously called bloodstream alanine-rich proteins), were 20-fold more abundant in salivary gland than midgut (procyclic) trypanosomes. Anti-BARP antisera reacted strongly and exclusively with salivary gland parasites and a BARP 3' flanking region directed epimastigote-specific expression of reporter genes in the fly, but inhibited expression in bloodstream and procyclic forms. In contrast to an earlier report, we could not detect BARPs in bloodstream forms. We propose that BARPs form a stage-specific coat for epimastigote forms and suggest renaming them *brucei* alanine-rich proteins.

14162. **Wang, Y., Singh, U. & Mueller, D. M., 2007**. Mitochondrial genome integrity mutations uncouple the yeast *Saccharomyces cerevisiae* ATP synthase. *Journal of Biological Chemistry*, **282** (11): 8228-8236.

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The mitochondrial ATP synthase is a molecular motor, which couples the flow of protons with phosphorylation of ADP. Rotation of the central stalk within the core of ATP synthase effects conformational changes in the active sites driving the synthesis of ATP. Mitochondrial genome integrity (mgi) mutations have been previously identified in the alpha-, beta-, and gamma-subunits of ATP synthase in yeast *Kluyveromyces lactis* and trypanosome *Trypanosoma brucei*. These mutations reverse the lethality of the loss of mitochondrial DNA

in petite negative strains. Introduction of the homologous mutations in *Saccharomyces cerevisiae* results in yeast strains that lose mitochondrial DNA at a high rate and accompanied decreases in the coupling of the ATP synthase. The structure of yeast F1-ATPase reveals that the mgi residues cluster around the gamma-subunit and selectively around the collar region of F1. These results indicate that residues within the mgi complementation group are necessary for efficient coupling of ATP synthase, possibly acting as a support to fix the axis of rotation of the central stalk.

14163. **Welburn, S. C., Macleod, E., Figarella, K. & Duzensko, M., 2006.** Programmed cell death in African trypanosomes. *Parasitology*, 132 Suppl: S7-S18.

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Until recently it had generally been assumed that apoptosis and other forms of programmed cell death evolved during evolution of the metazoans to regulate growth and development in these multicellular organisms. However, recent research is adding strength to the original phenotypic observations described almost a decade ago which indicated that some parasitic protozoa may have evolved a cell death pathway analogous to the process described as apoptosis in metazoa. Here we explore the implications of a programmed cell death pathway in the African tsetse-transmitted trypanosomes.

14164. **Willert, E. K., Fitzpatrick, R. & Phillips, M. A., 2007**. Allosteric regulation of an essential trypanosome polyamine biosynthetic enzyme by a catalytically dead homologue. *Proceedings of the National Academy of Sciences USA*, **104** (20): 8275-8280.

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African sleeping sickness is a fatal disease that is caused by the protozoan parasite *Trypanosoma brucei.* Polyamine biosynthesis is an essential pathway in the parasite and is a validated drug target for treatment of the disease. S-adenosylmethionine decarboxylase (AdoMetDC) catalyzes a key step in polyamine biosynthesis. Here, we show that trypanosomatids uniquely contain both a functional AdoMetDC and a paralogue designated prozyme that has lost catalytic activity. The *T. brucei* prozyme forms a high-affinity heterodimer with AdoMetDC that stimulates its activity by 1,200-fold. Both genes are expressed in *T. brucei*, and analysis of AdoMetDC activity in *T. brucei* extracts supports the finding that the heterodimer is the functional enzyme *in vivo*. Thus, prozyme has evolved to be a catalytically dead but allosterically active subunit of AdoMetDC, providing an example of how regulators of multimeric enzymes can evolve through gene duplication and mutational drift. These data identify a distinct mechanism for regulating AdoMetDC in the parasite that suggests new strategies for the development of parasite-specific inhibitors of the polyamine biosynthetic pathway.

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