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AFRICAN TRYPANOSOMES IN THE 21ST CENTURY: WHAT IS THEIR FUTURE IN SCIENCE AND IN HEALTH?

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The African trypanosomes remain well recognised for their role as an interesting model eukaryote for basic science, but are loosing ground in their ability to contribute to understanding common cellular mechanisms. At the same time, the diseases they cause remain as prevalent as ever but appear increasingly irrelevant in their wider medical, social, economic and political context. What can be done to keep trypanosome biology relevant and vigorous in the 21st century?

Key Words: African trypanosomes, Trypanosoma brucei, genomics, cooperation, future of research, disease.

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INTRODUCTION

"Strange to see how a good dinner and feasting reconciles everybody" Samuel Pepys, English diarist, 1665.

What is an 'Internet Conference' and can it be effective? I'm interpreting this invitation to 'deliver' a 'keynote address' in the spirit of the dictionary definition 'conference: an interchange of views'. I hope that the views I offer, which I do not claim exclusively, will stimulate interchange and will not be regarded as my immutable opinions. During the current election season in the USA, it seems that changing one's opinions is a political liability, whereas common sense dictates that opinions should change in response to reasoned argument and new discoveries.

In summarising some of my thoughts, I am approaching this assignment as a conversation, perhaps even more so than a live conference talk would allow, and not with the rigorous scholarship demanded of the printed word. I'll try to keep it brief. It remains for me to seen how such conversations work, although the internet appears to provide an increasing mode of 'conversation', at least in the realm of my 13-year-old daughter. Some of my remarks are intended to be provocative. They may even, occasionally, be considered offensive by some. If so, I apologise in advance, for no offense is intended. Maybe science sometimes needs its own internal 'shock radio' show, although I largely abhor that format and the often ignorant polarisation on which it feeds. The iconoclast, however, can play a useful role in the evolution of science.

I confess to some ambivalence about the role of this medium. This is the third trypanosome internet conference, but only the first in which several of the 'speakers' have participated. I did so after the exertion of an unusual degree of flattery and arm-twisting by the organisers, in the absence of the usual attractions of conferences held in exotic locations (see quotation above). The foundation of my support is that a virtual conference has the potential to be far more democratic than even the most democratic international conference in our field, the annual Woods Hole Molecular Parasitology meeting, which immediately preceded this internet event. Whether virtual conferences will have significant impact in the next few years is unclear, although the internet is a scientific tool without which most of us could not do effective research today. Communication is the lifeline of research and more is better.

A BRIEF HISTORY OF AFRICAN TRYPANOSOMES

Trypanosomatids are ubiquitous unicellular flagellated protozoa that are ancient survivors on planet Earth. They probably embarked on their own evolutionary branch more than 500 million years ago (Stevens and Gibson, 1999), prior to the origins of their present invertebrate and vertebrate hosts. At the turn of the century, epidemics of human sleeping sickness threatened to decimate equatorial Africa. Today, human trypanosomiasis is as prevalent as ever but its relative importance has been greatly diminished in comparison to HIV-induced decimation, amounting to millions of deaths per year in Africa, the epicentre of AIDS. Animal trypanosomiasis is still regarded as a major problem, causing economic, social and nutritional impact in Africa, but the presence of the animal reservoir and the Tsetse, the ever-present human to animal contact, and the breakdown of infrastructure in many parts of Africa, mean that human trypanosomiasis remains a serious threat (Barrett, 1999; Hide, 1999). Where does this leave the community of researchers in African trypanosomiasis? Speaking personally, it is somewhat depressing to be sidelined by relatively new diseases of vastly greater significance. I and, I suspect, many colleagues who entered this field at around the same time were optimistic that our work would lead to improved cures or prevention of typanosomiasis. Perhaps we were just very naïve. Even if our research led to new drugs, the social impact in Africa would be rather insignificant in a continent that appears increasingly disadvantaged by economic and political circumstances. According to an editorial in the July 15th 2000 issue of The Economist, the World Bank estimates that Africa alone may need to spend more than $2 billion per year to control AIDS. Although few of us, and certainly not I, can speak authoritatively to these wider issues, and without wishing to diminish local initiatives that are probably the most meaningful, one can hardly consider any infectious disease without recognising its social and political context. However, one only has to witness the local reaction to the appearance of West Nile Encephalitis virus in New York City to realize how poor is the perspective of the average US citizen, when it comes to being aware of the relative impact of infectious diseases and their geographical significance. Other diseases cause far more deaths in New York, guns about 100-fold more, in one of the safest American cities, but mosquitoes have

zero political clout! One suspects that the principal culprits of this emergent disease are the infected birds, whose flight range rather exceeds that of the lowly mosquito, but what mayor could dare suggest killing birds, or put reputation at risk by promising to eliminate guns! What local politician would dare compare the insignificant risk of West Nile encephalitis to what our fellow citizens of the world are exposed to in Africa, especially during an election campaign.

Having got NY politics out of my system, for the moment, I'll start the real 'address' by listing some of the key events in the early history of the African Trypanosomiasis, for those who don't know it. Then I will try to highlight prospects of research in (mainly African) trypanosomes, some of which will be discussed in more detail and with more authority by other contributors.

The first reports of human sleeping sickness were filed in 1803, although one assumes that the disease is far more ancient, perhaps ancient enough for it to have influenced human evolution, as have malaria and other infectious diseases. The evidence on which I base this statement comes from the genetics of the haptoglobin-related protein, which plays an as yet unexplained role in innate human resistance to *Trypanosoma brucei brucei* (Smith et al., 1995; Tomlinson and Raper, 1998; Molina Portela et al., 2000). Human primate ancestors may have evolved resistance to *T. brucei brucei* when the haptoglobin-related-protein (Hpr) arose by a triplication of the haptoglobin locus in old-world primates, subsequently reduced to a duplication in humans (McEvoy and Maeda, 1988). Intriguingly, the Hpr gene has been amplified in a significant proportion of African Americans (Maeda et al., 1986).

The first pathogenic trypanosome, *Trypanosoma evansi*, was discovered in the blood of equines and camels, in India, in 1880 and David Bruce discovered *Trypanosoma brucei* as the agent of the 'Tsetse fly disease' in African cattle in 1894 (Bruce, 1895). Trypanosomes were first demonstrated in human sleeping sickness in 1902 and experiments reported in 1909 described the first attempt at immunisation and the first demonstration of the innate resistance conferred by human serum (Laveran and Mesnil, 1902). In 1905, Franke and Ehrlich performed experiments from which they concluded that "… the trypanosomes must therefore have acquired other biological properties during their stay in [the monkey's] semi-immune body that rendered them resistant to the defensive substances", this providing the first intimation of the phenomenon of antigenic variation, which some of us are still trying to understand today. Kleine recognised the existence of a developmental cycle for *Trypanosoma brucei* in Glossina (Tsetse) in 1909. Suramin (Bayer 205) was discovered in 1917 (patented in 1924) and remains the primary treatment for acute human trypanosomiasis, despite its often serious side effects.

THE TRYPANOSOME AS A MODEL ORGANISM FOR CELL AND MOLECULAR BIOLOGY

What keeps many of us excited about the field is that trypanosomes have established themselves scientifically as the best studied example of a 'differently evolved' eukaryote, although other organisms may emerge to challenge this role. Trypanosomes offer an alternative life-style compared to organisms that are sometimes extolled as representing the conservation of key features 'throughout evolution', from yeast through fly to human. However, I think we are falling behind where we were a few years ago, relative to more 'mainstream' organisms. I will say more about this later.

How different are trypanosomes?

We already know of several areas of basic cell function where trypanosomes differ remarkably from their mammalian hosts. Paramount among these are RNA editing of mitochondrial genome transcripts (Simpson et al., 2000), the unique compartmentalisation of glycolysis in organelles that encapsulate other pathways that are common to peroxisomes, which glycosomes resemble in this and other respects (Michels et al., 2000), and the polycistronic and apparently unregulated transcription by RNA polymerase I. No factors that regulate transcription initiation have yet been discovered in trypanosomes. The translation machinery of the trypanosome mitochondrion must be one of the most primitive systems to which we have experimental access, but it has not yet been studied. Perhaps this is one area in which the genome project, coupled with the recent publication of high-resolution structures of bacterial ribosomal subunits (Agalarov et al., 2000; Ban et al., 2000), could spark renewed interest. I think it is still one of the unexplored frontiers of trypanosome research, although I've never worked in the area.

In what areas can trypanosomes continue to contribute?

What have trypanosomes contributed to the understanding of pathways that are shared, in at least some respects, by other cells? I hope I can say, without accusations of partisanship, that the major example has to be the pioneering work on protein glycosylphosphatidylinositol (GPI) anchoring. Although the core enzymes and genes responsible for the shared aspects of this pathway are now being rapidly identified in yeast and mammalian cells, there are still some interesting puzzles, such as the role of the GPI lipid in protein sorting during secretion and recycling, in which trypanosomes can probably play an illuminating role. It remains a mystery why trypanosomes are so particular about uniquely and exclusively incorporating myristate into their VSG anchor. There is something important here, lots of decent speculation, no specific evidence, but it almost certainly has something to do with endocytic sorting (Mukherjee et al., 1999). Because *Leishmania* can apparently survive the loss of all GPI-anchored surface proteins, it may offer unique genetic options for identifying genes involved in the GPI pathway (Hilley et al., 2000). Procyclic forms of *T. brucei* can also survive loss of all GPI-anchored proteins, but only under certain culture conditions (Nagamune et al., 2000).

Another area in which I believe trypanosomes could have much to contribute is in understanding mechanisms of gene expression that do not involve the regulation of transcription initiation, which appears to be 'the main event' for most genes in better studied but quite diverse systems. In contrast, there is no evidence for any regulation of transcription initiation by RNA Polymerases I or II in trypanosomes. My enthusiasm for this area is based on the assumption that an understanding of the (apparently simpler) ways in which trypanosomes regulate transcription will help illuminate the basic and possibly ancestral role of chromatin structure in regulating gene expression, and identify a minimum complement of components necessary for transcription in vivo. Few people are currently studying these aspects of trypanosome biology. Far more are studying RNA editing, which should be largely worked out in the next few years.

Some of our studies suggest that information contained in the coding region can largely determine the expression level of a protein, at the level of translation or later. This situation may be responsible for our so-far unsuccessful attempts to over-express many potentially interesting proteins or (hopefully dominant-negative) mutant forms thereof. Does anyone else have some experience that would solve this problem? We're working on it but don't have definitive answers.

WHAT ARE THE KEYS TO PROGRESS IN TRYPANOSOME RESEARCH?

Genome projects

Genome analysis is the most important thing happening, at the present time, for *T. brucei*, *T. cruzi* and *Leishmania major*. Although resisted by a few, these projects can be, as in all other organisms where they are in progress, one of the most enabling activities for the better design of future experimental research. Among eukaryotes, trypanosomes are almost ideal for genome analysis. Genes are tightly packed on the chromosomes; there is, so far, only one example of an intron (Mair et al., 2000), but it seems reasonable to assume there will be others; and genes appear to be orientated unidirectionally over long regions, although this organisation needs to be reviewed with care lest important genes be overlooked. There are, however, several negative issues and several important technical obstacles that need to be overcome if we are to maximise the impact of the genome projects. At the first level of analysis, it looks as if gene comparisons will not identify the functions of more than 30% of trypanosome genes. We will need to develop and apply new and possibly trypanosome-specific tools to provide a more useful first-line analysis and annotation of the genome. Even then, it seems likely that the most interesting genes, which regulate mechanisms that are uniquely trypanosome-specific, will be trypanosome-specific. Identifying the functions of novel genes, which may be critical for trypanosome-specific survival, will be a major challenge. On the other hand, it will be easy to identify homologues of many genes whose function is known in other organisms. Some of these genes may be vital to trypanosome-specific phenomena. For example, histone acetyl transferases and deacetylases and genes involved in recombination and chromosome repair may play a role in the phenomenon of antigenic variation. Indeed, it is quite unlikely that they will not. But, should we resist the temptation to experiment with these conserved components and accept the challenge of identifying the unique and process-specific regulators? For many of us, it is difficult to make such a choice. It is probably easier to write successful grant applications on what is half

known than what is unknown, and there is the increasingly insidious mechanism of determining promotion and appointment policies by publication analysis.

More tools needed!

I am going to raise the issue of new tool development to highlight two points. Firstly, that we still need new tools and, secondly, how can we develop these more efficiently when funding agencies do not normally hand out resources for tool development as such, because it is not viewed as 'hypothesis-driven' research. Consequently, most of us who have developed new tools have either had the benefit of serendipity (a classic example was recognising the potential significance of a small error that instantly led to the elimination of the 'feeder-cell' requirement for bloodstream-form culture (Duszenko et al., 1985)) or we've pursued it part time at the risk of not achieving the stated objectives of our funding. Sometimes, however, enlightened reviewers have acceded to small sections inserted into otherwise hypothesis-driven research proposals.

What we most lack in trypanosomes are techniques for 'traditional' or 'forward' genetics: finding or creating phenotype then identifying the responsible genes. Although we can perform 'reverse' genetics with sufficient efficiency that it could, theoretically, be scaled up to perform large-scale 'knockout' studies, both forward and reverse genetics suffer from some common problems. Paramount among these are that trypanosomes are diploid and remain quite tedious to grow under conditions where phenotypic screens can easily be performed. Indeed, what will people want to screen for and how? The value of 'real' genetics is very evident, from other systems, but designing the screens in trypanosomes is another significant challenge.

What possibilities are there for doing 'forward' genetics? For several years, we have made a part-time attempt to develop the Mariner transposition system (Gueiros-Filho and Beverley, 1997) for *T. brucei*. This now seems close to the level of efficiency where it could be applied to real-world gene identification, but how do we deal with diploidy? One possibility is to induce allelic conversion of the initially single-marker insertions by increasing drug pressure, but early attempts to do this, in carefully contrived situations, have not been encouraging (Clayton, 1999). Another suggestion that has been made is to use chemical mutagenesis on the single-allele Mariner insertion library. This looks like a good idea, but has not yet been put to the test.

Double-stranded or 'interfering' RNA, RNAi, has great potential for identifying the functions of genes where interference, at the mRNA level, will have rapid effects. Two recent reports show how effective this approach can be when a striking phenotype can be predicted on the basis of sequence motifs or other evidence (Shi et al., 2000; Wang et al., 2000). One major drawback in the action of RNAi in trypanosomes is the high amount of double stranded RNA that apparently has to be generated, which is in marked contrast to the situation in *Caenorhabditis elegans*, for example. My personal hope is that once the genes that are essential for the extreme activities of RNAi in other organisms have been identified, it may become possible to construct trypanosome lines that are vastly more sensitive to the effects of double-stranded RNA, thereby allowing large gene scans to be performed. In the meantime, however, conditional gene disruption offers an alternative to RNAi, in *T. brucei*, although it involves more manipulations (Wirtz et al., 1998; Wirtz et al., 1999).

Even with the six proven drug-resistance markers that work in *T. brucei*, there are limitations in reverse genetics that make the development of new approaches desirable. One of the most obvious tools we lack is a proven negative selectable marker that will permit the re-use of positive markers but also the ability to make single nucleotide changes to genes in situ. Although thymidine kinases that render *T. brucei* susceptible to antiviral analogues have been tested (Valdes et al., 1996; Cross et al., 1998), they have not yet found wide application. Introduction of cytosine deaminase might provide an alternative means of negative selection (Fox et al., 1999). Construction of strains that provide the opportunity to mimic the yeast URA3 system should be quite easy, but the problem that is always pointed out to me is that serum may be a sufficient source of pyrimidines to render an auxotroph viable. However, no-one has explored this experimentally.

Culture systems for trypanosomes still leave much room for improvement and, except for procyclic *T. brucei*, where growth in the absence of serum is possible (Cross and Manning, 1973), culture media are complex and undefined. Only two life-cycle stages of *T. brucei* can be grown in culture and the life cycle is generally regarded as a one-way street, although it can be reversed under rather tedious culture conditions (Hirumi et al., 1992). Even if the necessary conditions were not physiological, how much of a technical advantage it would be to be able to readily convert *T. brucei* from procyclic to infective mammalian forms. Few labs have access to the Tsetse and, even then, it is not a facile system.

THE DRUG PROBLEM

Drug problems come in many forms (a stew of science, economics, sociology and politics, once again). It is obvious that, despite some heroic efforts surrounding the introduction of DFMO (Schechter and Sjoerdsma, 1986; Pepin et al., 2000), we desperately need universally effective and safe drugs for all trypanosomatid infections: for multiple species of *Leishmania* and trypanosomes (Werbovetz, 2000). How do we achieve this noble aim with limited resources in the absence of commercial incentives?

Probably more than half of trypanosomatid research is justified on the basis of identifying 'new drug targets'. Much of this is lip service. There are, however, several important initiatives and several great prospects for drug development. What we desperately need are resources and commitment on a scale that can transform targets from the abstract to the actual. There are perhaps two ways in which these objectives can be best pursued. Neither is a novel. Firstly, new sources of serious funding have to be identified that are sufficient to encourage and subsidise the participation of the pharmaceutical industry, preferably in partnership with those who know the organisms, in screening for new trypanocides. Secondly, because of their different evolution, trypanosomes present a plethora of potential drug target, but we should currently encourage those that could ride on the back of pathways that are currently the target of huge efforts in the pharmaceutical industry. Among these one would obviously include topoisomerases (Nenortas et al., 1998) and protein prenylation (Yokoyama et al., 1998a; Yokoyama et al., 1998b), but maybe others can make a case for prioritising their pet pathways.

EVER A VACCINE?

Few researchers of African trypanosomes remain optimistic about vaccination. Antigenic variation represents a formidable obstacle that has apparently evolved to evade the immune response. Once we understand more about how antigenic variation works, or about intrinsic mechanisms that regulate trypanosome multiplication, it may be possible to envision strategies to intervene therapeutically in these processes. Immunisation with surface proteins other than the VSG have been discouraging, although few have been tested. The flagellar pocket remains an intriguing and possibly relevant target. It is possible that new vaccine candidates will emerge from the genome project and from refined methods for membrane separation and micro-scale protein identification. I'm seriously doubtful about this but would be pleased to hear any strong counter-arguments. Everyone would be delighted if a vaccination strategy could be developed.

COOPERATION VERSUS COMPETITION IN THE GENOMIC ERA

I would like to conclude with a few remarks about the way we operate, as researchers. We are a small community. Indeed, in Africa and the USA, the community of trypanosome researchers seems to have shrunk. In Europe the community appears stable or, in the UK, significantly larger than it was a decade ago.

As has happened in other fields of biology, large-scale technology, starting with genome projects, has focussed attention on the value of cooperation rather than competition, although a significant element of the latter persists at the core of some genomic studies.

I would like to invoke two parallels from computing, where one of the rallying cries these days is 'open source' software, another is 'cross-platform' applications. In biology, a genome sequence is the source and it is, or should be, open. However, the sequence is just a new beginning and most of it will not make sense at this stage. How can we encourage more open sourcing of work that adds value to the basic sequence? The other issue that concerns me is the plethora of 'strains' that are used in trypanosome

research. This can have advantages as well as disadvantages but shouldn't we at least try to focus our research on one or two strains, in the immediate future, at least where studies of gene function are concerned? While things that may be important may be missed if processes are studied in only one strain, there are also many examples where differing results from different labs can be hard to reconcile (even after applying Pepys' formula), when performed in different genetic backgrounds.

One of the key issues with African trypanosomes has been the issue of so-called 'monomorphic' versus 'pleomorphic' cell lines. Whereas monomorphic lines can be easily derived from pleomorphic ones, there seems to be an assumption, by many people, that the monomorphic phenotype represents an irreversible change in genotype. This has been a debate in which people have sometimes taken rather unscientific positions, as well as misrepresenting the other side. The real problem is sometimes, one suspects, emotional. The more rational problem is that many among us have made a great investment in analysing one particular strain. However, two or three strains have some overwhelming reasons why they should be the lines of choice, at least for research in the immediate future. Parenthetically, this comment obviously does not apply to lines that are studied for specific and biologically important phenotypes, such as human infectivity.

What lines should we focus on? Pleomorphic lines are far more difficult to grow as bloodstream-form cultures, or in animals. In neither situation can high yields be obtained, which are necessary for many biochemical and cell structure studies. Monomorphic lines, of which the infamous Lister 427 has probably been most widely used, may have lost their ability to revert to a pleomorphic phenotype, owing to their long history of sub-passaging in animals, but this has not been specifically tested. Lister 427 does have many advantages, however, for studying most aspects of trypanosome biology except the bloodstream pleomorphic transition. Despite many published misrepresentations, it does retain the ability to complete the life cycle in the Tsetse, as demonstrated by three independent laboratories (GAMC, manuscript in preparation), and the efficiency of this transition could probably be easily improved by performing more than one cycle of transmission to re-select for efficiently cycling lines. The strain chosen for the genome project, TREU927 is pleomorphic. Recent reports have shown that the parasitaemia to which it grows can be improved in a few subpassages, probably without an irreversible genotypic change, and that it can then be cultured and transfected (Christine Clayton, personal communication). For many studies, therefore, this may be the line of choice. One of the most important reasons is that if one is interested in a particular gene, the clone encoding that gene can be identified from the genome project and rapidly retrieved from a BAC library. This is one step shorter than with the 427 line, where two good BAC libraries exist and can be immediately obtained from the same source, but filters still need to be screened to identify the corresponding clone. How important is it to use genes cloned from the syngeneic cell line? So far we do not know the extent of polymorphisms between cell lines and whether this would be an obstacle to performing genetic experiments in a non syngeneic line: probably not, in most instances.

In the past, those closer to the real field were frequently to be heard making the point that *T. brucei* is far from the most important trypanosome in Africa, at least in the veterinary arena. So, the vast majority of us who work with *T. brucei* stand accused of already working with a largely irrelevant organism, whether it is pleomorphic or not! Am I the only person to have officially proposed that the genomes of *Trypanosoma congolense* and *Trypanosoma vivax* should be a high priority for sequencing, once *T. brucei* has been completed?

Even more cooperation and collaboration will be highly desirable in the postgenomics era. There will simply be so much more interesting science to be done that, put into perspective, we won't be able to exploit the genome without more cooperation. This is a political and economic fact. So far, in trypanosomes, we have (excluding VSG genes) only explored about 100 of the approximately 5,000 unique genes, and their corresponding products, that are encoded in trypanosome genomes. How are we to extend our analyses in a way that the promise of the genome projects, to lead to better designed research, will be fulfilled? I'm still lukewarm about the likely value of massive co-operative knockout studies, although such experiments are likely to be more efficiently performed on a large scale than hitherto. There are also the vast arenas of protein and mRNA analysis. The latter has the seductive advantage of being easy and relatively cheap to automate, in the form of 'array analysis', although it is as yet unclear how informative this will be in trypanosomes, where most regulation of gene expression appears to occur downstream of transcription, perhaps way downstream in many cases. My own belief is

that all of these methods will contribute to our overall understanding. Because many RNAs are probably regulated by turnover, it seems to me that, once available in a form in which all genes are simultaneously represented, array analysis will become as indispensable as Northern blots are today, and vastly more informative. One can easily see that every gene knockout or change in environmental conditions to which the cells are subjected would be informatively analysed at the level of the entire mRNA repertoire.

However, even before we get to postgenomics, there is an important issue of how to make the genomic data, and their offshoots, easily accessible and accurately represented. For this is will be important to have curated databases that talk to each other transparently. Probably not just one database in one place, because that puts too much responsibility and influence in one place, but interactive ones. Most of us are aware that one of the main problems in current first-level approaches to genome annotation is that it is largely hostage to the inconsistent way in which database entries have been annotated previously. Thus, one of the next most important functions of the genome project will be to provide mechanisms for reliable and consistent annotation of genes across all trypanosomatid species.

I seem to have exceeded the intended length of this essay without much discussion of issues surrounding the future of trypanosomes in public health, although there are hints about my inner thoughts in what I have written. I will end on the issue of collaboration versus competition. What can be done to encourage collaboration without loosing the benefits of competition? The main issues are egos and credit, and the effects that the assignment of priority has on peer recognition and funding decisions. These aspects are driven by competition and currently lack formal mechanisms for recognising the role of collaboration. What can we do to improve the mechanisms for assigning credit and making decisions on funding, so that we can effectively pursue the role of trypanosomes in science and in health, on what seems likely to be an inadequate budget to satisfy even modest aspirations?

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References

Agalarov, S. C., Sridhar Prasad, G., Funke, P. M., Stout, C. D., Williamson, J. R., 2000. Structure of the S15,S6,S18-rRNA complex: assembly of the 30S ribosome central domain. Science 288, 107-13.

Ban, N., Nissen, P., Hansen, J., Moore, P. B., Steitz, T. A., 2000. The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 289, 905-20.

Barrett, M. P., 1999. The fall and rise of sleeping sickness. Lancet 353, 1113-1114.

Bruce, D., 1895. Tsetse fly disease or nagana. Harrison & Sons, London.

Clayton, C. E., 1999. Genetic manipulation of kinetoplastida. Parasitol. Today 15, 372-378.

Cross, G. A. M., Manning, J. C., 1973. Cultivation of Trypanosoma brucei sspp. in semi-defined and defined media. Parasitology 67 , 315-331.

Cross, M., Taylor, M. C., Borst, P., 1998. Frequent loss of the active site during variant surface glycoprotein expression site switching in vitro in *Trypanosoma brucei*. Mol. Cell Biol. 18, 198-205.

Duszenko, M., Ferguson, M. A. J., Lamont, G. S., Cross, G. A. M., 1985. Cysteine eliminates the feeder cell requirement for cultivation of *Trypanosoma brucei* bloodstream forms in vitro. J. Exp. Med. 162, 1256-1263.

Fox, B. A., Belperron, A. A., Bzik, D. J., 1999. Stable transformation of *Toxoplasma gondii* based on a pyrimethamine resistant trifunctional dihydrofolate reductase-cytosine deaminase- thymidylate synthase gene that confers sensitivity to 5-fluorocytosine. Mol. Biochem. Parasitol. 98, 93-103.

Gueiros-Filho, F. J., Beverley, S. M., 1997. Trans-kingdom transposition of the *Drosophila* element mariner within the protozoan *Leishmania*. Science 276, 1716-1719.

Hide, G., 1999. History of sleeping sickness in East Africa. Clin. Microbiol. Rev. 12, 112-25.

Hilley, J. D., Zawadzki, J. L., McConville, M. J., Coombs, G. H., Mottram, J. C., 2000. *Leishmania mexicana* mutants lacking glycosylphosphatidylinositol (GPI) protein transamidase provide insights into the biosynthesis and functions of GPI-anchored proteins. Mol. Biol. Cell 11, 1183-1195.

Hirumi, H., Hirumi, K., Moloo, S. K., Shaw, M. K., 1992. *Trypanosoma brucei brucei*: in vitro production of metacyclic forms. J. Protozool. 39, 619-627.

Laveran, A., Mesnil, F., 1902. Recherches sur le traitement et la prevention du nagana. Annales de L'Institut Pasteur 16, 785-817.

Maeda, N., McEvoy, S. M., Harris, H. F., Huisman, T. H., Smithies, O., 1986. Polymorphisms in the human haptoglobin gene cluster: chromosomes with multiple haptoglobin-related (Hpr) genes. Proc. Natl. Acad. Sci. U S A 83, 7395-9.

Mair, G., Shi, H. F., Li, H. J., Djikeng, A., Aviles, H. O., Bishop, J. R., Falcone, F. H., Gavrilescu, C., Montgomery, J. L., Santori, M. I., Stern, L. S., Wang, Z. F., Ullu, E., Tschudi, C., 2000. A new twist in trypanosome RNA metabolism: cis-splicing of pre-mRNA. RNA-A Publication of the RNA Society 6, 163-169.

McEvoy, S. M., Maeda, N., 1988. Complex events in the evolution of the haptoglobin gene cluster in primates. J. Biol. Chem. 263, 15740-7.

Michels, P. A. M., Hannaert, V., Bringaud, F., 2000. Metabolic aspects of glycosomes in trypanosomatidae - New data and views. Parasitol. Today 16, 482-489.

Molina Portela, M. P., Raper, J., Tomlinson, S., 2000. An investigation into the mechanism of trypanosome lysis by human serum factors. Mol. Biochem. Parasitol. 110, 273-82.

Mukherjee, S., Soe, T. T., Maxfield, F. R., 1999. Endocytic sorting of lipid analogues differing solely in the chemistry of their hydrophobic tails. J. Cell Biol. 144, 1271-84.

Nagamune, K., Nozaki, T., Maeda, Y., Ohishi, K., Fukuma, T., Hara, T., Schwarz, R. T., Sutterlin, C., Brun, R., Riezman, H., Kinoshita, T., 2000. Critical roles of glycosylphosphatidylinositol for *Trypanosoma brucei*. Proc. Natl. Acad. Sci. USA 97, 10336-10341.

Nenortas, E. C., Bodley, A. L., Shapiro, T. A., 1998. DNA topoisomerases - a new twist for antiparasitic chemotherapy. Biochimica et Biophysica Acta - Gene Structure & Expression 1400, 349-354.

Pepin, J., Khonde, N., Maiso, F., Doua, F., Jaffar, S., Ngampo, S., Mpia, B., Mbulamberi, D., Kuzoe, F., 2000. Short-course eflornithine in Gambian trypanosomiasis: a multicentre randomized controlled trial. Bulletin of the World Health Organization 78, 1284-1295.

Schechter, P. J., Sjoerdsma, A., 1986. Difluoromethylornithine in the treatment of African Trypanosomiasis. Parasitol. Today 2 , 223-224.

Shi, H. F., Djikeng, A., Mark, T., Wirtz, E., Tschudi, C., Ullu, E., 2000. Genetic interference in *Trypanosoma brucei* by heritable and inducible double-stranded RNA. RNA-A Publication of the RNA Society 6, 1069-1076.

Simpson, L., Thiemann, O. H., Savill, N. J., Alfonzo, J. D., Maslov, D. A., 2000. Evolution of RNA editing in trypanosome mitochondria. Proc. Natl. Acad. Sci. USA 97, 6986-6993.

Smith, A. B., Esko, J. D., Hajduk, S. L., 1995. Killing of trypanosomes by the human haptoglobin-related protein. Science 268, 284-286.

Stevens, J. R., Gibson, W., 1999. The molecular evolution of trypanosomes. Parasitol. Today 15, 432-437.

Tomlinson, S., Raper, J., 1998. Natural human immunity to trypanosomes. Parasitol. Today 14, 354-359.

Valdes, J., Taylor, M. C., Cross, M. A., Ligtenberg, M. J. L., Rudenko, G., Borst, P., 1996. The viral thymidine kinase gene as a tool for the study of mutagenesis in *Trypanosoma brucei*. Nucleic Acids Res. 24, 1809-1815.

Wang, Z., Morris, J. C., Drew, M. E., Englund, P. T., 2000. Inhibition of *Trypanosoma brucei* gene expression by RNA interference using an integratable vector with opposing T7 promoters. J. Biol. Chem. 275, 40174-40179.

Werbovetz, K. A., 2000. Target-based drug discovery for malaria, leishmaniasis, and trypanosomiasis. Current Medicinal Chemistry 7, 835-860.

Wirtz, E., Hoek, M., Cross, G. A. M., 1998. Regulated processive transcription of chromatin by T7 RNA polymerase in *Trypanosoma brucei*. Nucleic Acids Res. 26, 4626-4634.

Wirtz, E., Leal, S., Ochatt, C., Cross, G. A. M., 1999. A tightly regulated inducible expression system for dominant negative approaches in *Trypanosoma brucei*. Mol. Biochem. Parasitol. 99, 89-101.

Yokoyama, K., Trobridge, P., Buckner, F. S., Scholten, J., Stuart, K. D., Van Voorhis, W. C., Gelb, M. H., 1998a. The effects of protein farnesyltransferase inhibitors on trypanosomatids: inhibition of protein farnesylation and cell growth. Mol. Biochem. Parasitol. 94, 87-97.

Yokoyama, K., Trobridge, P., Buckner, F. S., Vanvoorhis, W. C., Stuart, K. D., Gelb, M. H., 1998b. Protein farnesyltransferase from *Trypanosoma brucei* : a heterodimer of 61- and 65-kda subunits as a new target for antiparasite therapeutics. J. Biol. Chem. 273, 26497-26505.