View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Chalmers Publication Library

CHALMERS

Chalmers Publication Library

Trypanosoma brucei: meet the system

This document has been downloaded from Chalmers Publication Library (CPL). It is the author´s version of a work that was accepted for publication in:

Current Opinion in Microbiology (ISSN: 1369-5274)

Citation for the published paper:
Achcar, F. ; Kerkhoven, E. ; Barrett, M. (2014) "Trypanosoma brucei: meet the system". Current Opinion in Microbiology, vol. 20 pp. 162-169.

http://dx.doi.org/10.1016/j.mib.2014.06.007

Downloaded from: http://publications.lib.chalmers.se/publication/204748

Notice: Changes introduced as a result of publishing processes such as copy-editing and formatting may not be reflected in this document. For a definitive version of this work, please refer to the published source. Please note that access to the published version might require a subscription.

Chalmers Publication Library (CPL) offers the possibility of retrieving research publications produced at Chalmers University of Technology. It covers all types of publications: articles, dissertations, licentiate theses, masters theses, conference papers, reports etc. Since 2006 it is the official tool for Chalmers official publication statistics. To ensure that Chalmers research results are disseminated as widely as possible, an Open Access Policy has been adopted.
The CPL service is administrated and maintained by Chalmers Library.

(article starts on next page)

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Trypanosoma brucei: meet the system

Fiona Achcar[1], Eduard J Kerkhoven[2] and Michael P Barrett[1]

African trypanosomes cause devastating diseases in humans and domestic animals. The parasites evolved early in the eukaryotic lineage and have numerous biochemical peculiarities that distinguish them from other systems. These include unconventional mechanisms for expressing nuclear and mitochondrial genes as well as unusual subcellular localizations for a variety of enzymes. Systems biology has arisen partly to allow contextualization of the massive datasets that describe individual chemical parts of biological systems. Here we describe recent efforts to collect and analyse data pertaining to all aspects of the trypanosome's biochemical physiology that go some way to describing the parasite as an integrated system.

Addresses
[1] Wellcome Trust Centre of Molecular Parasitology and Glasgow Polyomics, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, United Kingdom
[2] Systems and Synthetic Biology, Department of Chemical and Biological Engineering, Chalmers University of Technology, Kemivägen 10, SE-412 96 Göteborg, Sweden

Corresponding author: Barrett, Michael P (michael.barrett@glasgow.ac.uk)

Current Opinion in Microbiology 2014, **20**:162–169

This review comes from a themed issue on **Host–microbe interactions: parasites**

Edited by **Manoj Duraisingh** and **Nancy Guillén**

For a complete overview see the Issue and the Editorial

Available online 16th July 2014

http://dx.doi.org/10.1016/j.mib.2014.06.007

1369-5274/Published by Elsevier Ltd.

Human African trypanosomiasis

African trypanosomes of the *Trypanosoma brucei* group cause human African trypanosomiasis, a disease of sub-Saharan Africa whose distribution is limited by the ecological range of the tsetse fly vectors that transmit these parasites [1].

T. brucei gambiense and *T. b. rhodesiense* cause chronic and acute forms of the disease, respectively. After a tsetse bite, trypanosomes enter the blood and lymphatic system. When non human-infectious African trypanosomes enter the bloodstream, lipoprotein particles enter through endocytosis after binding a haptoglobin like receptor, then lyse due to trypanosome lytic factors (TLFs) including Apolipoprotein L1 [2]. *T. b. rhodesiense* avoids lysis through its serum resistance associated (SRA) protein, a mutated version of a variant surface glycoprotein (VSG), that binds and neutralises ApoL1 [2]. *T. b. gambiense* express reduced levels of the haptoglobin-like receptor [3] and also express TgsGP [4•,5], another VSG derivative that protects against lysis by modifying membrane fluidity. People carrying rare mutations in ApoL1 become susceptible to other species of trypanosome, such as *Trypanosoma evansi* [6,7].

In humans, trypanosomes establish chronic infections by varying expression of the thousand or so genes that encode different coat glycoproteins [8]. These shroud the parasite and exclude immune effectors from the cell surface. Antibodies against these coat proteins eventually induce complement mediated lysis of parasites. However, cells expressing other coat variants escape and proliferate until new antibodies are generated. Eventually, parasites invade the central nervous system triggering stage 2 disease, characterized by progressive deterioration of brain function leading to death. Metacyclic forms injected by the tsetse fly are non-proliferative and these differentiate into slender bloodstream forms, adapted to live in the haemolymphatic system and later cerebrospinal fluid (CSF). A quorum-sensing pathway [9•] triggers differentiation of slender forms into a non-proliferative stumpy form whose biochemistry is pre-adapted for survival in the tsetse midgut. Here, stumpy forms differentiate into replicative procyclic forms that eventually, after transforming through several other forms, become the metacyclic forms pre-adapted biochemically for the mammalian host.

The parasite

Trypanosomes belong to the order Kinetoplastida, named after the kinetoplast, a dense intercatenated network of circular mitochondrial DNA molecules (kDNA). The genetic code of kDNA is perturbed such that genes are transcribed into RNA molecules that must be edited into translatable mRNA through the addition or removal of U-residues [10]. The expression of nuclear genes is also unconventional (see later).

Other unusual cellular phenomena in trypanosomes include a sub-pellicular microtubule array that maintains cell morphology. Various organelles include acidocalcisomes, involved in ion and pH homeostasis. Peroxisomes of trypanosomes are highly adapted containing the first seven enzymes of glycolysis, hence their being named 'glycosomes'. Redox balance involves two glutathione molecules complexed to spermidine to create the signature metabolite, trypanothione (N^1,N^8-bis(glutathionyl)-spermidine). Much effort has focused on attempting to

target these unusual features with chemicals that could become new drugs.

The trypanosome's nuclear genome and gene expression

T. brucei is diploid, possessing around 10,000 nuclear, protein-coding genes across 11 pairs of classical chromosomes in the megabase range (0.9–5.7 Mb) [11]. They also contain variable numbers of intermediate (0.3–0.9 Mb) and mini chromosomes (0.05–0.1 Mb) as VSG gene repositories [12]. Other genes are present in single or multiple copies, often in tandem arrays. Some correlation exists between gene copy number and transcription level. Next Generation Sequencing technologies have allowed comparison between different species [13], subspecies and strains and efforts are underway to compare trypanosomes that are, for example, responsible for different pathologies, host-range specificity and other phenotypic traits.

VSG gene transcription involves an RNA polymerase 1-containing extranucleolar expression site body (ESB) that ensures the monoallelic expression of VSGs, since only one vsg gene can be associated with the ESB at a time [14].

Elsewhere, large parts of the genome are transcribed constitutively and expression is regulated primarily at the level of RNA stability and translational control. The transcription of genes encoding the main surface proteins of procyclic forms, called procyclins, is also PolI dependent. Transcription of other protein coding genes is dependent on polymerase II, although PolII promoters have not been found.

Different genes give rise to transcripts and proteins of different abundance throughout the life cycle. For example, the bloodstream form specific glucose transporter gene, THT1, is found only in bloodstream form (BSF) and this is also reflected in steady-state RNA levels [15]. Conversely, the pentose phosphate pathway enzyme transketolase is procyclic specific [16]. Several genome-wide transcription studies have been performed [17–20]. Meta-analysis of this data identified co-expression networks of genes and some overlap with similar networks from the related parasite *Leishmania infantum*, providing clues on functional assignment by association [19]. Expression analysis also revealed the mechanistic pathways involved in differentiation, for example the transcription of a phosphatase encoding gene, whose expression is pivotal in transformation to stumpy forms, was markedly upregulated [21]. Carboxylic acid transporters associated with the stumpy to procyclic transformation [22] were also identified through differentiation-linked transcription of these genes.

As transcription is constitutive, large polycistronic transcripts must be processed by the combined action of polyadenylation at the 3′ end of individual genes and addition of a spliced leader sequence at the 5′ end [23] (Figure 1A).

Regulatory elements within the 3′ untranslated region (3′UTR) of transcripts can determine stability of the message or translation efficiency. RNA-binding proteins of different classes (RRM, ALBA, CCCH and puf families) [24] have all been characterized. One member, RBP10, was shown to be key in controlling bloodstream form specific gene expression [25] and its phosphorylation seems to associate with its control function. Another protein, RBP6, was found to be highly expressed in parasites within the tsetse fly proventiculus [26••]. Expressing RBP6 in procyclics was sufficient to stimulate their transformation to mammal-infective metacyclics expressing VSG [26••]. RNA binding proteins are pivotal, therefore, in determining expression of families of genes in pre-programmed pathways.

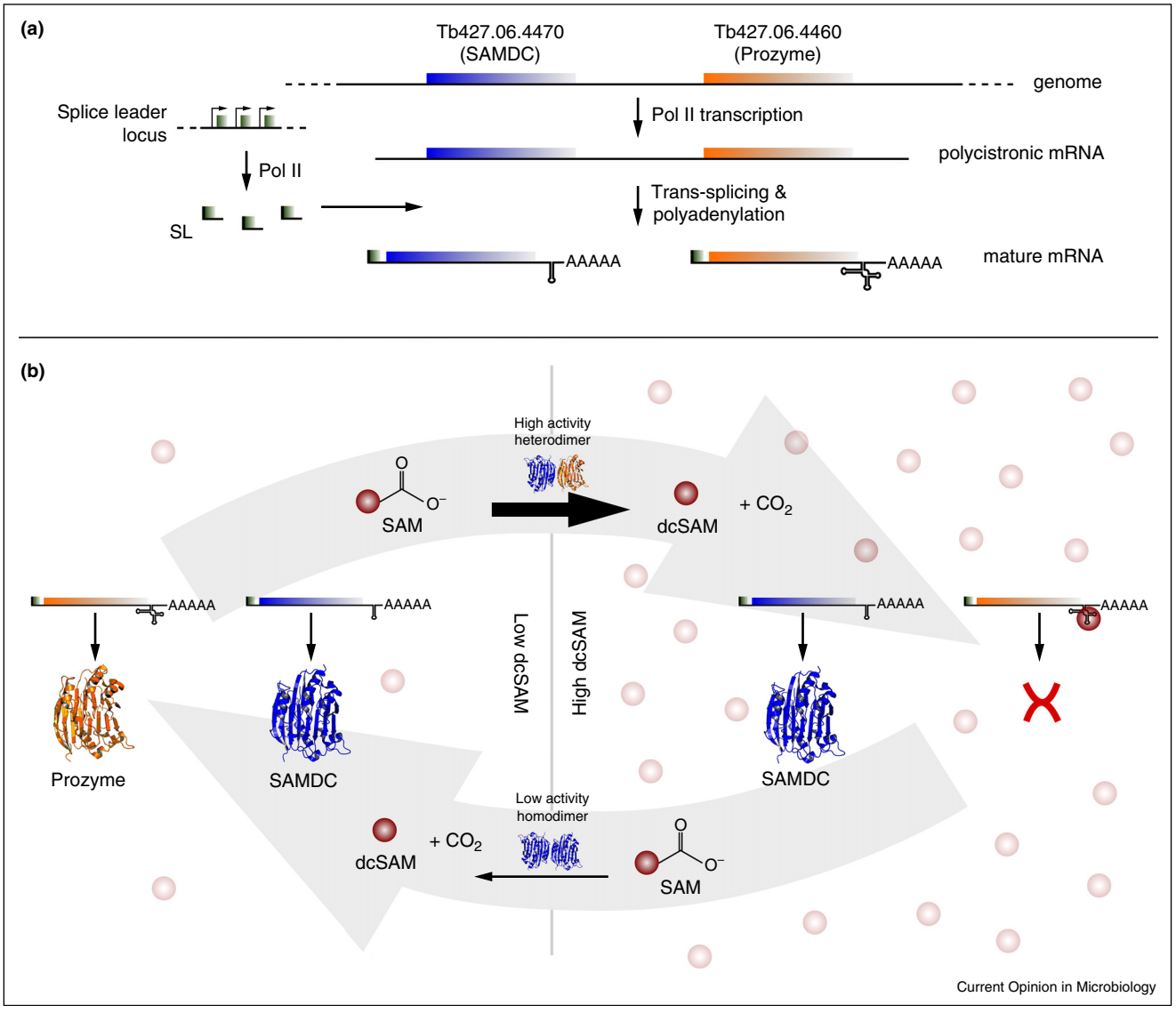
Other genes and pathways are under environmental control. For example, proline is the usual substrate for energy metabolism in tsetse flies, but procyclic trypanosomes prefer glucose if available [27]. Threonine's use in acetate production is also regulated based on glucose availability [28].

Polyamine homeostasis is of particular importance. Tight regulation occurs and the metabolite, decarboxylated S-adenosylmethionine (dcSAM) plays a central role. S-adenosylmethionine decarboxylase (SAMDC), which converts SAM to dcSAM [29], is activated over 100 fold when bound to a catalytically dead homologue called prozyme [30••]. Prozyme's RNA has a region within its 3'UTR that is proposed to bind to dcSAM (Figure 1B). When that metabolite is abundant, translation of the message arrests. As dcSAM levels fall, the unbound message is translated, creating more prozyme. dcSAM then rises, binds to the mRNA and represses translation again. Such feedback systems involving metabolite, protein and message offer exquisite balance of metabolism and understanding such regulation is the essence of modern systems biology. The discovery that a weakly active deoxyhypusine synthase also associates with an inactivate paralogue, encoded by a separate gene, to create a highly active heterodimer [31•], points to our only just beginning to unravel much of the complexity of the trypanosome system.

Genome-wide functional genomics using RNA interference

T. brucei possesses the machinery for RNA interference. Initially the technique was used to knockdown individual genes through creation of double stranded RNA and several efforts have been taken to assess whole classes of genes e.g. the kinome [32]. It has become possible to systematically knockdown expression of all genes using genome-wide libraries of fragments expressed as dsRNA

Figure 1



Regulation of polyamine biosynthesis through a putative decarboxylated S-adenosylmethionine riboswitch. **(a)** Adjacent genes encode S-adenosylmethionine decarboxylase (SAMDC) and an enzymatically dead paralogue, termed prozyme. Note the trans-splicing and polyadenylation that process individual trypanosome mRNAs from large polycistronic precursors. **(b)** Enzyme activity is high when prozyme binds to SAMDC. Prozyme translation from its mRNA, however, seems to depend upon the cellular abundance of dcSAM (depicted as red circles here). If abundant, the metabolite binds to a region of the 3′UTR of the transcript and blocks translation. This reduces the level of prozyme, thus reducing activity of SAMDC and ultimately reducing the levels of dcSAM. As the metabolite levels fall, the 3′UTR region of prozyme transcript loses bound metabolite. This allows translation to start again, providing a typical metabolic feedback loop.

in Sca1 meganuclease expressing *T. brucei* lines (which have greatly improved transfection efficiency). Initially by transfecting parasites with the library which was sequenced before and after propagation in trypanosomes, hundreds of genes whose loss affected growth rates across their life cycle were detected [33]. The technique was then adapted for positive selection for any genes whose loss of function rendered parasites less susceptible to trypanocidal drugs [34••]. Most recently genes whose loss of function prevent parasites differentiating in response to non-metabolisable cAMP analogues into growth-arrested stumpy forms have been determined too [9•].

Proteomics

Proteins provide both the structural cornerstone defining cellular form and also the catalytic capability defining function. Proteomics allows quantification of individual

protein levels within the system and the relative proteomes of different trypanosome life cycle stages have been assessed [35]. Methods also exist to locate proteins in different organelles, and several studies have aimed to catalogue the glycosomal sub-proteome [36,37], which has multiple pathways beyond glycolysis including the pentose phosphate pathway, nucleotide salvage, pyrimidine biosynthesis, arginine kinase, a succinate shunt and several aspects of lipid metabolism among others. Assembly of proteins into complexes with different partners is also crucial to cellular function — and delineation of the trypanosome's protein complexes is underway. A multi-enzyme complex specific to the trypanosome system is the mitochondrial editosome, the complex machinery driving RNA editing [10].

Mass spectrometry is also able to identify post-translational modifications to proteins and the phosphoproteomes of bloodstream form and procyclic forms were compared [38,39]. More finely tuned alterations in real time signalling cascades might also be possible.

Metabolomics

Mass spectrometry (MS), or nuclear magnetic resonance (NMR), based methods now allow quantification of the small molecule component of cells and systems. Metabolomics has recently been applied to *T. brucei* [40]. For example, experiments where drugs were applied to trypanosomes have lead to identification of their targets. Eflornithine provoked a dramatic increase in the abundance of cellular ornithine and corresponding decrease in putrescine, the substrate and product, respectively, of the drug's target, ornithine decarboxylase [41••]. Metabolomics also revealed that trypanosomes use few nutrients present in rich media used in their cultivation [42]. Heavy atom labeled substrates allow the tracing of molecules throughout the metabolic network and increasingly studies of this type are being applied to different systems including trypanosomes and other parasites. These types of analysis will revolutionise our understanding of the small molecule composition of living systems and greatly facilitate our ability to follow the transformations that underlie the building of organisms. For example, the apicomplexan parasites *Toxoplasma gondii* [43] and *Plasmodium falciparum* [44] were shown to produce γ-aminobutyric acid (GABA) from glutamine by following the fate of the ^{13}C-heavy atom labeled carbon from U-^{13}C-glutamine. It was subsequently possible to demonstrate genes encoding enzymes of GABA shunt in these parasites. In *T. brucei* using glucose of which half was the U-^{13}C-glucose derivative showed how this substrate partitioned into many parts of metabolism including a glycosomal succinate shunt (since succinate, fumarate and malate could all be identified [45] with three of their four carbons labeled, whilst TCA cycle derived versions of these metabolites would be expected to have either two or four labeled carbons being derived from acetate). Unusual metabolites such as octuolse 8-phosphate derived from the pentose phosphate pathway were also identified. Recent data indicates that bloodstream forms too might have a more extensive glucose metabolism than previously considered.

In addition to labeling in the steady state, tracer experiments can also be used to determine fluxes through the system and, increasingly, these approaches will be used to learn about the pathways involved in creating the trypanosomal system. Metabolomics has also been used to determine how trypanosomes affect the host plasma and urine metabolomes [46].

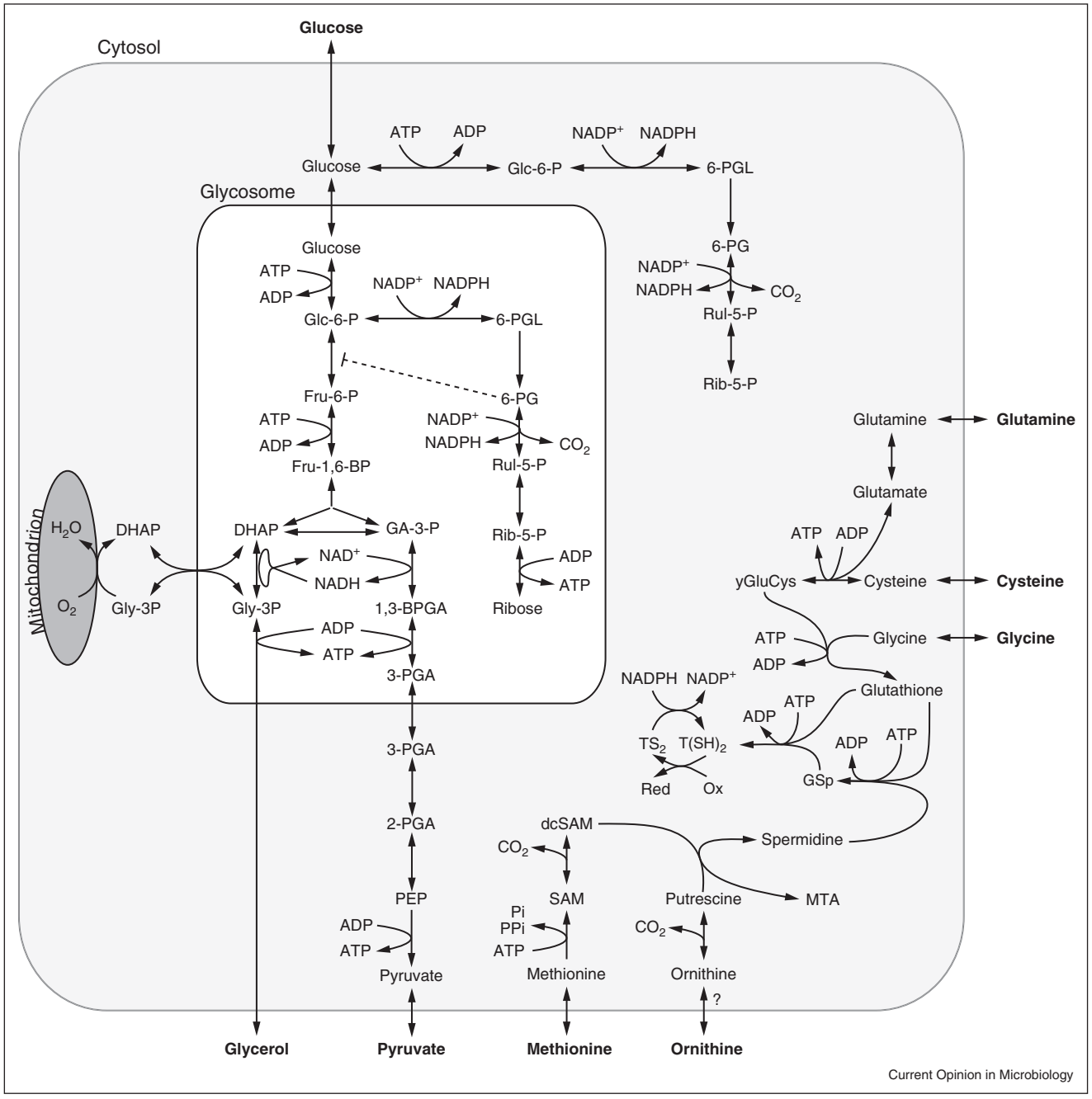
Mathematical modelling of the trypanosomal system

Computational models of various aspects of the trypanosomal system have been developed. Ambitious efforts to model growth of parasites in the mammalian bloodstream have been employed [47,48] where parasite intrinsic factors (antigenic variation and quorum sensing growth arrest) work along with host factors including immune response to create the characteristic undulating parasitaemia.

Metabolism has also been modelled. Coarse-grained static models of metabolism have been generated based on pathways inferred from genome-wide analysis of enzymes predicted to be present e.g. KEGG and a community-based Metacyc related project, termed trypanocyc (http://metdev.toulouse.inra.fr/). This information will eventually be incorporated into a constraint-based model allowing predictions of metabolic flux through the networks as previously attempted for *Leishmania* [49] and *Trypanosoma cruzi* [50].

Dynamic models of key parts of trypanosome metabolism have also been developed. The first seven enzymes of glycolysis are localized to the glycosome. Kinetic parameters for all of the enzymes from glucose transport, through hexokinase, to pyruvate kinase and the pyruvate transporter are available. A series of ordinary differential equations incorporating these kinetic parameters was created [51] and flux of glucose to pyruvate successfully simulated. The model has been updated repeatedly as new information comes to light and made fascinating predictions on novel ways to kill trypanosomes by provoking differentiation at an inappropriate time [52]. An interesting effort to include the production and stability of mRNA and protein turnover for phosphoglycerate kinase attempted to introduce multi-layered modelling into the flow of information [53]. Most recently, algorithms permitting inclusion of uncertainty about the system, including ranges of kinetic parameters reflecting different parameters measured in different labs, rather than single representative values, have been introduced [54]. The topology that describes the distribution of metabolites between compartments is also uncertain, given the technical difficulties in precise quantification within different compartments, and by including this

Figure 2



Glucose and thiol metabolism modelled in *Trypanosoma brucei*. Mathematical models have been produced that depict the catabolism of glucose via the glycolytic pathway in the glycosome and also the pentose phosphate pathway which is dually localized to the glycosome and the cytosol. The polyamine and glutathione/trypanothione pathways have also been modelled mathematically and can be linked to glycolysis and the PPP via NADPH, a key reductant generated by the PPP and consumed via trypanothione reductase to maintain this pivotal thiol in its reduced form such that it can operate to maintain cellular redox balance.

uncertainty [55] (allowing a fraction of each enzyme to be active in both cytosol and glycosome) allowed glycolysis to run even with the presence of recently described metabolite-permeability pores in the glycosomal membrane [56] (Figure 2).

The presence of enzymes of a second key pathway of glucose catabolism, the pentose phosphate pathway (PPP), in both the glycosome and the cytosol, added more complexity but a model capable of predicting flux through both branches was generated. The diversion of

bound phosphate from glucose 6-phosphate into the PPP breaks the proposed bound-phosphate pool in the glycosome [57•]. Additional reactions must protect against this. RNAi of ribokinase ruled out this enzyme taking such a role alone, but a network of other reactions preserving bound phosphate could be operative.

Other basic kinetic models, this time of the polyamine and trypanothione pathways, have also been attempted for *T. brucei* [58] and *T. cruzi* [59]. In principle, such models could be refined and combined with the existing glycolysis-pentose phosphate pathway model via the cofactor NADPH which is created by the pentose phosphate pathway and is critical to the functioning of the trypanothione system.

Conclusions

Progress is being made in understanding the trypanosomal cell from the systems perspective, which combines information from all levels into an operative unit. The challenges towards this are large. However, as creation of the system becomes increasingly tangible we can also look ahead to the host–parasite combined system and in the same way begin to learn how the host attempts to control the parasite and how the parasite deals with host molecular mechanisms to eliminate it.

Acknowledgments

EJK was funded by The Scottish Universities Life Sciences Alliance. We are grateful to SysMO for funding the SilicoTryp (project: http://www.sysmo.net/index.php?index=164). The Wellcome Trust Centre for Molecular Parasitology is supported by core funding from the Wellcome Trust (085349).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Brun R, Blum J: **Human African trypanosomiasis**. *Infect Dis Clin North Am* 2012, **26**:261-273.

2. Stephens NA, Kieft R, Macleod A, Hajduk SL: **Trypanosome resistance to human innate immunity: targeting Achilles' heel**. *Trends Parasitol* 2012, **28**:539-545.

3. Kieft R, Capewell P, Turner CM, Veitch NJ, MacLeod A, Hajduk S: **Mechanism of *Trypanosoma brucei gambiense* (group 1) resistance to human trypanosome lytic factor**. *Proc Natl Acad Sci U S A* 2010, **107**:16137-16141.

4. • Uzureau P, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homblé F, Grélard A, Zhendre V, Nolan DP, Lins L, Crowet JM, Pays A, Felu C, Poelvoorde P, Vanhollebeke B, Moestrup SK, Lyngsø J, Pedersen JS, Mottram JC, Dufourc EJ, Pérez-Morga D, Pays E: **Mechanism of *Trypanosoma brucei gambiense* resistance to human serum**. *Nature* 2013, **501**:430-434.

A gene unique to *T. b. gambiense*, TgsGP, was shown to be capable of conferring resistance to human serum. It is therefore analogous to the sra gene previously described in *T. b. rhodesiense*, although mechanisms of resistance are different.

5. Capewell P, Clucas C, DeJesus E, Kieft R, Hajduk S, Veitch N, Steketee PC, Cooper A, Weir W, MacLeod A: **The TgsGP gene is essential for resistance to human serum in *Trypanosoma brucei gambiense***. *PLOS Pathog* 2013, **9**:e1003686.

6. Vanhollebeke B, Truc P, Poelvoorde P, Pays A, Joshi PP, Katti R, Jannin JG, Pays E: **Human *Trypanosoma evansi* infection linked to a lack of apolipoprotein L-I**. *N Engl J Med* 2006, **355**:2752-2756.

7. Truc P, Büscher P, Cuny G, Gonzatti MI, Jannin J, Joshi P, Juyal P, Lun ZR, Mattioli R, Pays E, Simarro PP, Teixeira MM, Touratier L, Vincendeau P, Desquesnes M: **Atypical human infections by animal trypanosomes**. *PLOS Negl Trop Dis* 2013, **7**:e2256.

8. Glover L, Hutchinson S, Alsford S, McCulloch R, Field MC, Horn D: **Antigenic variation in African trypanosomes: the importance of chromosomal and nuclear context in VSG expression control**. *Cell Microbiol* 2013, **15**:1984-1989.

9. • Mony BM, MacGregor P, Ivens A, Rojas F, Cowton A, Young J, Horn D, Matthews K: **Genome-wide dissection of the quorum sensing signalling pathway in *Trypanosoma brucei***. *Nature* 2014, **505**:681-685.

The RITseq approach, where libraries of DNA fragments allow genome wide knockdown of all genes in the trypanosome genome was used to dissect the pathways involved in trypanosome differentiation from slender to stumpy forms.

10. Göringer HU: **'Gestalt' composition and function of the *Trypanosoma brucei* editosome**. *Annu Rev Microbiol* 2012, **66**:65-82.

11. Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, Lennard NJ, Caler E, Hamlin NE, Haas B, Böhme U, Hannick L, Aslett MA, Shallom J, Marcello L, Hou L, Wickstead B, Alsmark UC, Arrowsmith C, Atkin RJ, Barron AJ, Bringaud F, Brooks K, Carrington M, Cherevach I, Chillingworth TJ, Churcher C, Clark LN, Corton CH, Cronin A, Davies RM, Doggett J, Djikeng A, Feldblyum T, Field MC, Fraser A, Goodhead I, Hance Z, Harper D, Harris BR, Hauser H, Hostetler J, Ivens A, Jagels K, Johnson D, Johnson J, Jones K, Kerhornou AX, Koo H, Larke N, Landfear S, Larkin C, Leech V, Line A, Lord A, Macleod A, Mooney PJ, Moule S, Martin DM, Morgan GW, Mungall K, Norbertczak H, Ormond D, Pai G, Peacock CS, Peterson J, Quail MA, Rabbinowitsch E, Rajandream MA, Reitter C, Salzberg SL, Sanders M, Schobel S, Sharp S, Simmonds M, Simpson AJ, Tallon L, Turner CM, Tait A, Tivey AR, Van Aken S, Walker D, Wanless D, Wang S, White B, White O, Whitehead S, Woodward J, Wortman J, Adams MD, Embley TM, Gull K, Ullu E, Barry JD, Fairlamb AH, Opperdoes F, Barrell BG, Donelson JE, Hall N, Fraser CM, Melville SE, El-Sayed NM: **The genome of the African trypanosome *Trypanosoma brucei***. *Science* 2005, **309**:416-422.

12. Rudenko G: **African trypanosomes: the genome and adaptations for immune evasion**. *Essays Biochem* 2011, **51**:47-62.

13. Jackson AP, Berry A, Aslett M, Allison HC, Burton P, Vavrova-Anderson J, Brown R, Browne H, Corton N, Hauser H, Gamble J, Gilderthorp R, Marcello L, McQuillan J, Otto TD, Quail MA, Sanders MJ, van Tonder A, Ginger ML, Field MC, Barry JD, Hertz-Fowler C, Berriman M: **Antigenic diversity is generated by distinct evolutionary mechanisms in African trypanosome species**. *Proc Natl Acad Sci U S A* 2012, **109**:3416-3421.

14. Navarro M, Gull K: **A pol I transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei***. *Nature* 2001, **414**:759-763.

15. Bringaud F, Baltz T: **Differential regulation of two distinct families of glucose transporter genes in *Trypanosoma brucei***. *Mol Cell Biol* 1993, **13**:1146-1154.

16. Stoffel SA, Alibu VP, Hubert J, Ebikeme C, Portais JC, Bringaud F, Schweingruber ME, Barrett MP: **Transketolase in *Trypanosoma brucei***. *Mol Biochem Parasitol* 2011, **179**:1-7.

17. Archer SK, Inchaustegui D, Queiroz R, Clayton C: **The cell cycle regulated transcriptome of *Trypanosoma brucei***. *PLOS Pathog* 2011, **6**:e18425.

18. Kolev NG, Franklin JB, Carmi S, Shi H, Michaeli S, Tschudi C: **The transcriptome of the human pathogen *Trypanosoma brucei* at single-nucleotide resolution**. *PLOS Pathog* 2010, **6**:e1001090.

19. Najafabadi HS, Lu Z, MacPherson C, Mehta V, Adoue V, Pastinen T, Salavati R: **Global identification of conserved post-transcriptional regulatory programs in trypanosomatids**. *Nucleic Acids Res* 2013, **41**:8591-8600.

20. Choi J, El-Sayed NM: **Functional genomics of trypanosomatids**. *Parasite Immunol* 2012, **34**:72-79.

21. Szöor B, Wilson J, McElhinney H, Tabernero L, Matthews KR: **Protein tyrosine phosphatase TbPTP1: a molecular switch controlling life cycle differentiation in trypanosomes**. *J Cell Biol* 2006, **175**:293-303.

22. Dean S, Marchetti R, Kirk K, Matthews KR: **A surface transporter family conveys the trypanosome differentiation signal**. *Nature* 2009, **459**:213-217.

23. De Gaudenzi JG, Noé G, Campo VA, Frasch AC, Cassola A: **Gene expression regulation in trypanosomatids**. *Essays Biochem* 2011, **51**:31-46.

24. Kolev NG, Ullu E, Tschudi C: **The emerging role of RNA-binding proteins in the life cycle of *Trypanosoma brucei***. *Cellular Microbiology* 2014, **16**:482-489 http://dx.doi.org/10.1111/cmi.12268.

25. Wurst M, Seliger B, Jha BA, Klein C, Queiroz R, Clayton C: **Expression of the RNA recognition motif protein RBP10 promotes a bloodstream-form transcript pattern in *Trypanosoma brucei***. *Mol Microbiol* 2012, **83**:1048-1063.

26. •• Kolev NG, Ramey-Butler K, Cross GA, Ullu E, Tschudi C: **Developmental progression to infectivity in *Trypanosoma brucei* triggered by an RNA-binding protein**. *Science* 2012, **338**:1352-1353.

A single RNA binding protein was shown to trigger differentiation of procyclic to mammalian infectious metacyclic forms when expressed in the procyclics.

27. Bringaud F, Barrett MP, Zilberstein D: **Multiple roles of proline transport and metabolism in trypanosomatids**. *Front Biosci* 2012, **17**:349-374.

28. Millerioux Y, Ebikeme C, Biran M, Morand P, Bouyssou G, Vincent IM, Mazet M, Riviere L, Franconi JM, Burchmore RJ, Moreau P, Barrett MP, Bringaud F: **The threonine degradation pathway of the *Trypanosoma brucei* procyclic form: the main carbon source for lipid biosynthesis is under metabolic control**. *Mol Microbiol* 2013, **90**:114-129.

29. Willert E, Phillips MA: **Regulation and function of polyamines in African trypanosomes**. *Trends Parasitol* 2012, **28**:66-72.

30. •• Xiao Y, Nguyen S, Kim SH, Volkov OA, Tu BP, Phillips MA: **Product feedback regulation implicated in translational control of the *Trypanosoma brucei* S-adenosylmethionine decarboxylase regulatory subunit prozyme**. *Mol Microbiol* 2013, **88**:846-861.

A region within the 3′UTR of the transcript of the gene encoding prozyme was shown to regulate translation of transcript to protein depending on concentration of decarboxylated S-adenosylmethionine. As the metabolite levels drop, translation is triggered, increasing metabolite thus creating a regulatory feedback loop.

31. • Nguyen S, Jones DC, Wyllie S, Fairlamb AH, Phillips MA: **Allosteric activation of trypanosomatid deoxyhypusine synthase by a catalytically dead paralog**. *J Biol Chem* 2013, **288**:15256-15267.

The identification of a second instance where a catalytically dead paralogue of an enzyme, deoxyhypusine synthase, regulates activity of the enzyme through heterodimer formation. The other example is the S-adenosylmethionine decarboxylase-prozyme heterodimer.

32. Jones NG, Thomas EB, Brown E, Dickens NJ, Hammarton TC, Mottram JC: **Regulators of *Trypanosoma brucei* cell cycle progression and differentiation identified using a kinome-wide RNAi screen**. *PLOS Pathog* 2014, **10**:e1003886.

33. Alsford S, Turner DJ, Obado SO, Sanchez-Flores A, Glover L, Berriman M, Hertz-Fowler C, Horn D: **High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome**. *Genome Res* 2011, **21**:915-924.

34. •• Alsford S, Eckert S, Baker N, Glover L, Sanchez-Flores A, Leung KF, Turner DJ, Field MC, Berriman M, Horn D: **High-throughput decoding of antitrypanosomal drug efficacy and resistance**. *Nature* 2012, **482**:232-236.

Here the RITseq approach was used to systematically identify all genes whose loss of function causes resistance to the drugs used to treat trypanosomiasis.

35. Butter F, Bucerius F, Michel M, Cicova Z, Mann M, Janzen CJ: **Comparative proteomics of two life cycle stages of stable isotope-labeled *Trypanosoma brucei* reveals novel components of the parasite's host adaptation machinery**. *Mol Cell Proteomics* 2013, **12**:172-179.

36. Colasante C, Ellis M, Ruppert T, Voncken F: **Comparative proteomics of glycosomes from bloodstream form and procyclic culture form *Trypanosoma brucei* brucei**. *Proteomics* 2006, **6**:3275-3293.

37. Opperdoes FR, Szikora JP: **In silico prediction of the glycosomal enzymes of Leishmania major and trypanosomes**. *Mol Biochem Parasitol* 2006, **147**:193-206.

38. Urbaniak MD, Guther ML, Ferguson MA: **Comparative SILAC proteomic analysis of *Trypanosoma brucei* bloodstream and procyclic lifecycle stages**. *PLOS ONE* 2012, **7**:e36619.

39. Urbaniak MD, Martin DM, Ferguson MA: **Global quantitative SILAC phosphoproteomics reveals differential phosphorylation is widespread between the procyclic and bloodstream form lifecycle stages of *Trypanosoma brucei***. *J Proteome Res* 2013, **12**:2233-2244.

40. Creek DJ, Anderson J, McConville MJ, Barrett MP: **Metabolomic analysis of trypanosomatid protozoa**. *Mol Biochem Parasitol* 2012, **181**:73-84.

41. •• Vincent IM, Creek DJ, Burgess K, Woods DJ, Burchmore RJ, Barrett MP: **Untargeted metabolomics reveals a lack of synergy between nifurtimox and eflornithine against *Trypanosoma brucei***. *PLOS Negl Trop Dis* 2012, **6**:e1618.

The simultaneous identification of many hundreds of small molecule metabolites revealed that eflornithine is a highly selective inhibitor of ornithine decarboxylase.

42. Creek DJ, Nijagal B, Kim DH, Rojas F, Matthews KR, Barrett MP: **Metabolomics guides rational development of a simplified cell culture medium for drug screening against *Trypanosoma brucei***. *Antimicrob Agents Chemother* 2013, **57**:2768-2779.

43. MacRae JI, Sheiner L, Nahid A, Tonkin C, Striepen B, McConville MJ: **Mitochondrial metabolism of glucose and glutamine is required for intracellular growth of *Toxoplasma gondii***. *Cell Host Microbe* 2012, **12**:682-692.

44. MacRae JI, Dixon MW, Dearnley MK, Chua HH, Chambers JM, Kenny S, Bottova I, Tilley L, McConville MJ: **Mitochondrial metabolism of sexual and asexual blood stages of the malaria parasite *Plasmodium falciparum***. *BMC Biol* 2013, **11**:67.

45. Creek DJ, Chokkathukalam A, Jankevics A, Burgess KE, Breitling R, Barrett MP: **Stable isotope-assisted metabolomics for network-wide metabolic pathway elucidation**. *Anal Chem* 2012, **84**:8442-8447.

46. Li JV, Saric J, Wang Y, Utzinger J, Holmes E, Balmer O: **Metabonomic investigation of single and multiple strain *Trypanosoma brucei* brucei infections**. *Am J Trop Med Hyg* 2011, **84**:91-98.

47. MacGregor P, Szöőr B, Savill NJ, Matthews KR: **Trypanosomal immune evasion, chronicity and transmission: an elegant balancing act**. *Nat Rev Microbiol* 2012, **10**:431-438.

48. Gjini E, Haydon DT, Barry JD, Cobbold CA: **Critical interplay between parasite differentiation, host immunity, and antigenic variation in trypanosome infections**. *Am Nat* 2010, **176**:424-439.

49. Chavali AK, Blazier AS, Tlaxca JL, Jensen PA, Pearson RD, Papin JA: **Metabolic network analysis predicts efficacy of FDA-approved drugs targeting the causative agent of a neglected tropical disease**. *BMC Syst Biol* 2012, **6**:27.

50. Roberts SB, Robichaux JL, Chavali AK, Manque PA, Lee V, Lara AM, Papin JA, Buck GA: **Proteomic and network analysis characterize stage-specific metabolism in *Trypanosoma cruzi***. *BMC Syst Biol* 2009, **3**:52.

51. Bakker BM, Michels PA, Opperdoes FR, Westerhoff HV: **Glycolysis in bloodstream form *Trypanosoma brucei* can be understood in terms of the kinetics of the glycolytic enzymes**. *J Biol Chem* 1997, **272**:3207-3215.

52. Haanstra JR, Kerkhoven EJ, van Tuijl A, Blits M, Wurst M, van Nuland R, Albert MA, Michels PA, Bouwman J, Clayton C, Westerhoff HV, Bakker BM: **A domino effect in drug action: from metabolic assault towards parasite differentiation**. *Mol Microbiol* 2011, **79**:94-108.

53. Haanstra JR, Stewart M, Luu VD, van Tuijl A, Westerhoff HV, Clayton C, Bakker BM: **Control and regulation of gene expression: quantitative analysis of the expression of phosphoglycerate kinase in bloodstream form *Trypanosoma brucei***. *J Biol Chem* 2008, **283**:2495-2507.

54. Achcar F, Kerkhoven EJ, SilicoTryp Consortium, Bakker BM, Barrett MP, Breitling R: **Dynamic modelling under uncertainty: the case of *Trypanosoma brucei* energy metabolism**. *PLOS Comput Biol* 2012, **8**:e1002352.

55. Achcar F, Barrett MP, Breitling R: **Explicit consideration of topological and parameter uncertainty gives new insights into a well-established model of glycolysis**. *FEBS J* 2013, **280**:4640-4650.

56. Gualdron-López M, Vapola MH, Miinalainen IJ, Hiltunen JK, Michels PA, Antonenkov VD: **Channel-forming activities in the glycosomal fraction from the bloodstream form of *Trypanosoma brucei***. *PLOS Comput Biol* 2012, **7**:e34530.

57. • Kerkhoven EJ, Achcar F, Alibu VP, Burchmore RJ, Gilbert IH, Trybiło M, Driessen NN, Gilbert D, Breitling R, Bakker BM, Barrett MP: **Handling uncertainty in dynamic models: the pentose phosphate pathway in *Trypanosoma brucei***. *PLOS Comput Biol* 2013, **9**:e1003371.
Mathematical modelling, with the inclusion of parameters that account for uncertainty associated with experimental observations of the system was used to extend the mathematical of glycolysis to include the pentose phosphate pathway.

58. Gu X, Reid D, Higham DJ, Gilbert D: **Mathematical modelling of polyamine metabolism in bloodstream-form *Trypanosoma brucei*: an application to drug target identification**. *PLOS ONE* 2013, **8**:e53734.

59. Olin-Sandoval V, González-Chávez Z, Berzunza-Cruz M, Martínez I, Jasso-Chávez R, Becker I, Espinoza B, Moreno-Sánchez R, Saavedra E: **Drug target validation of the trypanothione pathway enzymes through metabolic modelling**. *FEBS J* 2012, **279**:1811-1820.