DER CLOËTTA-PREIS 2001
WIRD VERLIEHEN AN

FRAU PROFESSOR DR.

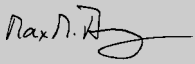
ISABEL RODITI

GEBOREN 1956 IN HARARE (ZIMBABWE),
PROFESSORIN FÜR ZELLBIOLOGIE AN DER UNIVERSITÄT BERN,
FÜR IHRE HERVORRAGENDEN ARBEITEN
AUF DEM GEBIETDER MOLEKULAREN PARASITOLOGIE.

ZÜRICH, 28. NOVEMBER 2001

FÜR DEN STIFTUNGSRAT:

DER PRÄSIDENT



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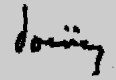

EIN MITGLIED

Prof. Isabel Roditi

CURRICULUM VITAE

Name:	Isabel Roditi
Date and place of birth:	5 October 1956, Harare, Zimbabwe
Nationality:	British

Higher Education:

1975 – 1979	B.A. (Hons) Biochemistry Wadham College, Oxford University, U.K.
1979 – 1982	Ph.D. Wolfson College and Division of Virology, Dept. of Pathology Cambridge University, U.K. Ph.D. thesis: «Cloning and Nucleotide Sequence Analysis of the Influenza Virus Genome»

Professional Experience:

Jan. 1983 – Sept. 1985	Post-doctoral Scientist MRC Biochemical Parasitology Unit Molteno Institute, Cambridge University, U.K.
Dec.1983 – March1984	Visiting Scientist ILRAD, Nairobi

Oct. 1985 – Dec. 1988	Post-doctoral Scientist
Jan. 1989 – Dec. 1989	Scientist Institut für Genetik und Toxikologie Kernforschungszentrum / Karlsruhe University, Germany
Jan. 1990 – Aug. 1999	Group leader / Titular professor Institut für Allgemeine Mikrobiologie University of Bern, Switzerland
1993	Habilitationsschrift: Stage-specific gene expression in African trypanosomes
March 1996	Promotion to titular Professor
Sept. 1999 –	Full professor Institut für Zellbiologie (formerly Institut für Allgemeine Mikrobiologie), University of Bern, Switzerland

GRANTS

Schweizerischer Nationalfonds (2001–)
Regulation of stage-specific gene expression and function of the surface coat of African trypanosomes

Wellcome Trust (2001–)
Collaborative grant with Dr L. Tetley and Prof J. D. Barry
The developmental fate of trypanosome surface coat mutants during tsetse transmission

Schweizerischer Nationalfonds (1998–2001)
Regulation of stage-specific gene expression in African trypanosomes

Stiftung 3R (1998–2000)
Transgenic protozoa as an alternative to transgenic animals

Stanley Thomas Johnson Foundation (1998–2002)
A novel approach to screening for drugs against African Sleeping Sickness

Roche Research Foundation and Novartis Foundation (1999)

Wellcome Trust Travel Award (1996–1999)
Collaborative grant with Prof J. D. Barry to study procyclin function

Novartis (1997/98)
Support for the 14th and 15th Swiss Trypanosomatid Meetings

Schweizerischer Nationalfonds (1995–1997)
Regulation of stage-specific gene expression in African trypanosomes

Helmut Horten Förderpreis (1993–1998)
Stage-specific expression and function of procyclins in African trypanosomes

Brachet Stiftung (1993–1996)
Collaborative grant with Prof T. Seebeck (Bern) and Prof E. Pays (Brussels) to study trypanosome differentiation

Schweizerischer Nationalfonds (Principal investigator Prof R. Braun, (1993–1996)
Genome organisation and gene expression of the protozoan parasite *Eimeria*

Schweizerischer Nationalfonds (1991–1994)
Function and Expression of Procyclin in African Trypanosomes

Schweizerischer Nationalfonds (1991–1994)
Molecular Biology of Coccidia *(Eimeria)* Parasites

ADDITIONALACTIVITIES

- Forschungsrätin, Schweizerischer Nationalfonds, Division IV

- Executive board, Faculty of Natural Sciences, University of Bern

- External reviewer for:
  Schweizerischer Nationalfonds
  Deutsche Forschungsgemeinschaft
  Belgian National Science Foundation
  Wellcome Trust

- Co-president of the Swiss Chapter of EWISH (European Women in Science and the Humanities) 1994–97

- Representative for Biology, University of Bern: The Situation of the Mittelbau at Swiss Universities (a study jointly commissioned by the Schweizerische Hochschulkonferenz, Schweizerischer Nationalfonds and Schweizerischer Wissenschaftsrat)

KEY PUBLICATIONS

01. Roditi, I., Carrington, M. & Turner, M. (1987). Expression of a polypeptide containing a dipeptide repeat is confined to the insect stage of *Trypanosoma brucei.* Nature 325, 272–74.

02. Dobbelaere, D. A. E., Coquerelle, T. M., Roditi, I. J. & Williams, R. O. (1988). *Theileria parva* infection induces autocrine growth of bovine lymphocytes. Proc. Nat. Acad. Sci. USA 85, 4730–4734.

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04. Pays, E., Coquelet, H., Tebabi, P., Pays, A., Jeffries, D., Steinert, M., Koenig, E., Williams, R. O. & Roditi, I. (1990). *Trypanosoma brucei:* constitutive activity of the VSG and procyclin promoters. EMBO J. 9, 3145–3151.

05. Freymann, D., Down, J., Carrington, M., Roditi, I., Turner, M & Wiley, D. (1990). A 2.9 Å resolution structure of the N-terminal domain of a variant surface glycoprotein from *Trypanosoma brucei.* J. Mol. Biol. 216, 141–160.

06. Hehl, A., Vassella, E., Braun, R. & Roditi, I. (1994). A conserved stem-loop structure in the 3' untranslated region of procyclin mRNAs regulates expression in *Trypanosoma brucei.* Proc. Natl. Acad. Sci. USA, 91, 370–374.

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10. Bütikofer, P., Vassella, E., Ruepp, S., Boschung, M., Civenni, G., Seebeck, T., Hemphill, A., Mookherjee, N., Pearson, T.W. and Roditi, I., (1999). Phosphorylation of a major GPI-anchored surface protein of *Trypanosoma brucei* during transport to the plasma membrane. J. Cell Sci., 112, 1785–1795.

11. Vassella, E., Van Den Abbeele, J., Bütikofer, P., Kunz Renggli, C, Furger, A., Brun, R. and Roditi I. (2000). A major surface glycoprotein of *Trypanosoma brucei* is expressed transiently during development and can be regulated post-transcriptionally by glycerol or hypoxia. Genes & Dev., 14, 615–626.

12. Acosta-Serrano, A., Vassella, E., Liniger, M., Kunz Renggli, C., Brun, R., Roditi, I. and Englund, P. (2001). The surface coat of procyclic *Trypanosoma brucei:* programmed expression and proteolytic cleavage of procyclin in the tsetse fly. Proc. Natl. Acad. Sci. USA, 98, 1513–1518.

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THE SURFACE COAT OF AFRICAN TRYPANOSOMES

Isabel Roditi

Preamble

If God had amused himself inventing the lilies of the field, he surely knocked His own socks off with the African parasites.

Barbara Kingsolver
The Poisonwood Bible

Anyone seriously committed to working with tropical parasites is likely to develop a love-hate relationship with them. They are beautiful, but deadly, and they alternately fascinate and frustrate us. Important phenomena such as antigenic variation (1), *trans*-splicing (2), RNAediting (3) and GPI anchoring (4, 5) would presumably have been discovered even if African trypanosomes did not exist, but they might have taken a lot longer. On the down side, there has been an explosive rise in the incidence of human sleeping sickness (current estimates lie between 300–500 thousand cases per year), there is no vaccine and the pharmaceutical industry has no interest in developing new drugs for an impoverished market. Ironically, despite their resurgence in Africa, trypanosomes are rapidly becoming an endangered species at Swiss universities, apart from a small endemic focus in the Bern-Fribourg area.

David Bruce, nagana and «fly disease»

Sie bewegten sich sehr lebhaft, meist wackelnd, doch auch bestimmt lokomotiv. Sah man einige Zeit zu, so kam ein heller Schwanz zum Vorschein, und später enthüllte sich allmählig ein längliches Thier...

G. Valentin, 1841

It may not be common knowledge that the first description of a trypanosome had its origins in Switzerland more than a hundred and fifty years ago. Gabriel Valentin, a professor of anatomy at the University of Bern, was examining a trout that had died from a neck wound when he noticed a small organism swimming between the blood cells. Valentin was sufficiently captivated by the sight of it to publish his observations, complete with illustrations, in 1841 (6). The name trypanosome, from the Greek words trypanon (drill) and soma (body) was coined two years later by David Gruby to describe similar parasites in the blood of frogs.

Fifty years after Valentin's publication, Surgeon-Major David Bruce persuaded the British Army to send him to South Africa to investigate the cause of the cattle disease known locally as nagana. The word nagana comes from the Zulu language and means «unwel» or «low in spirits». Bruce was an experienced microbiologist who had spent some time in Robert Koch's laboratory and had already identified the first pathogen to bear his name, *Brucella melitensis,* the bacterium causing Malta fever (also known as undulating fever). On arriving in South Africa Bruce set up a laboratory in Ubombo in Zululand and, within an astonishingly short time, succeeded in identifying the parasite now known as *Trypanosoma brucei* in the blood of infected animals. The trypanosomes could be transmitted to dogs and caused a fulminant parasitaemia with the same symptoms as nagana. Having solved this problem, Bruce then turned his attention to an apparently unrelated cattle disease occurring in the lowlands. The inhabitants of the area knew that «fly disease» was caused by tsetse flies, and that it tended to occur in regions where wild game were present, but it was thought to be due to a poison that was injected by the fly at the time of the bite. To his surprise, Bruce found trypanosomes in the blood of cattle with fly disease and was able to produce the same infection in dogs (7). A survey of game animals in the area revealed that many of them carried the parasites in their blood and it was realised that these were the reservoir for the disease. Bruce's proposed solution to the problem was draconian – shoot all wild game – which he justified on the grounds that «it would be as reasonable to allow mad dogs to live and to be protected by law in our English towns and villages».

For almost fifteen years after discovering the link between nagana and «fly disease», David Bruce was convinced that trypanosomes were mechanically transmitted by tsetse flies, even though Robert Koch had argued that the number of parasites in the blood of game animals was too low to permit transmission without an amplification step in the insect (8). Bruce had indeed observed trypanosomes in the tsetse fly midgut, but these were not infectious to animals (and thus did not fulfil Koch's postulates) and were regarded as degenerating bloodstream forms. The reason that the existence of a developmental cycle in the fly escaped notice for such a long time was due to the fact that transmission of *T. brucei* is an extremely inefficient process. In the wild, in contrast to optimised laboratory conditions, only a very small number of flies carry mature infectious forms of the parasite. This finding was finally published in the Proceedings of the Royal Society in 1909 (9), in a paper that should be mandatory reading for every scientist. First of all, it starts with the most masterly opening sentence that I have ever read in a scientific paper (Fig. 1) and, like many of Bruce's publications, it is superbly illustrated by his wife, Mary Bruce.

The following experiment is so complete in itself
that no apology is offered for publishing it by itself...

Figure 1. Illustration by Mary Bruce from the publication «The Development of *Trypanosoma gambiense* in *Glossina palpalis*» in 1909. The text is the opening sentence of the paper.

The link to human sleeping sickness

At the time of the arrival of the [sleeping sickness] commission, Dr Castellani did not consider that this trypanosome had any causal relationship to the disease, but thought it was an accidental concomitant like *Filaria perstans* ...

David Bruce

There are two forms of human sleeping sickness: a chronic form caused by *T. brucei gambiense* is prevalent in West Africa, and an acute form caused by *T. brucei rhodesiense* occurs in the south-east of the continent. Hundreds of years before the identification of African trypanosomes, slave-traders in West Africa were aware that slaves with grossly swollen lymph nodes at the back of the neck (Winterbottom's sign; Fig. 2) were very likely to die on the crossing to America and ten ded to exclude them from the «cargo». The early symptoms of the disease include waves of fever, swollen lymph nodes, headaches and joint pains. In the later phase the parasites invade the central nervous system causing neurological damage which can manifest itself in personality changes, changes in sleep patterns, seizures and finally coma. The disease is invariably fatal if untreated.

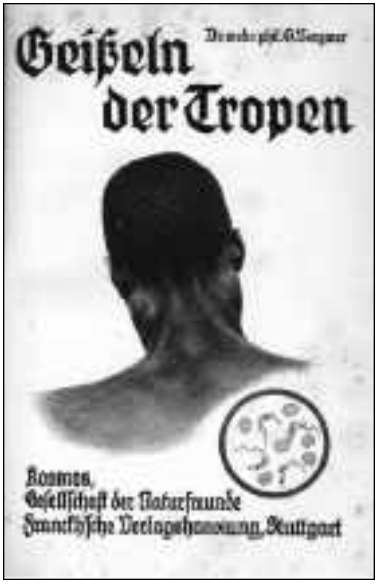
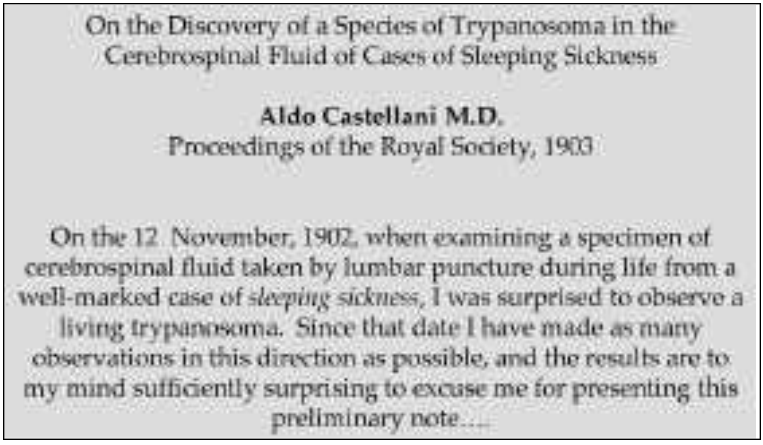


Figure 2. Frontispiece from «Geisseln der Tropen» with an example of Winterbottom's sign.

The identification of trypanosomes as the causative agents of sleeping sickness at the beginning of the twentieth century was as controversial

as the identification of HIV as the causative agent of AIDS at the end of the century. Officially the credit goes to Aldo Castellani (Fig. 3) but, according to Bruce, Castellani was originally of the opinion that the disease was caused by *Streptococcus* and that the trypanosomes were due to an incidental infection. After discussing this with Bruce, and apparently being convinced that there might be an alternative explana-


On the Discovery of a Species of Trypanosoma in the Cerebrospinal Fluid of Cases of Sleeping Sickness

Aldo Castellani M.D.
Proceedings of the Royal Society, 1903

On the 12 November, 1902, when examining a specimen of cerebrospinal fluid taken by lumbar puncture during life from a well-marked case of *sleeping sickness*, I was surprised to observe a living trypanosoma. Since that date I have made as many observations in this direction as possible, and the results are to my mind sufficiently surprising to excuse me for presenting this preliminary note....

Figure 3. The first report of a possible connection between trypanosomes and human sleeping sickness.

tion, Castellani then insisted that his findings be kept secret and that he should be the only author of the report linking trypanosomes to sleeping sickness (10). Was Bruce really setting the record straight, as he claimed several years later, or was he merely peeved that he had not made the discovery himself? Whatever the answer, cut-throat competition in science is nothing new, it seems.

The next point was to establish whether human and animal trypanosomiases were caused by the same species of parasite. Why, for example, were there no cases of sleeping sickness in some areas where nagana was rampant in cattle? One approach to the problem was to isolate trypanosomes from animals and to inoculate two volunteers. When these failed to cause an infection, a further 129 human guinea pigs were

enlisted – this in an era when there was not a single drug that could cure the disease. Fortunately, not one of them became infected, because, as we know now, only *T. b. brucei* is killed by trypanolytic factors in human serum (11, 12); the other two members of the «brucei» group, *T. b. gambiense* and *T. b. rhodesiense* infect both humans and animals. In addition, as will be explained below, the borderline between human serum-resistant and serum-sensitive strains is much more diffuse than was previously realised.

Life cycle

Much of the research in my laboratory is concerned with the trypanosome's transition from the mammalian host to the tsetse fly and its expression of surface molecules that are specific for different life cycle stages. For this reason the reader will not be spared a fairly detailed description of the parasite life cycle (Fig. 4).

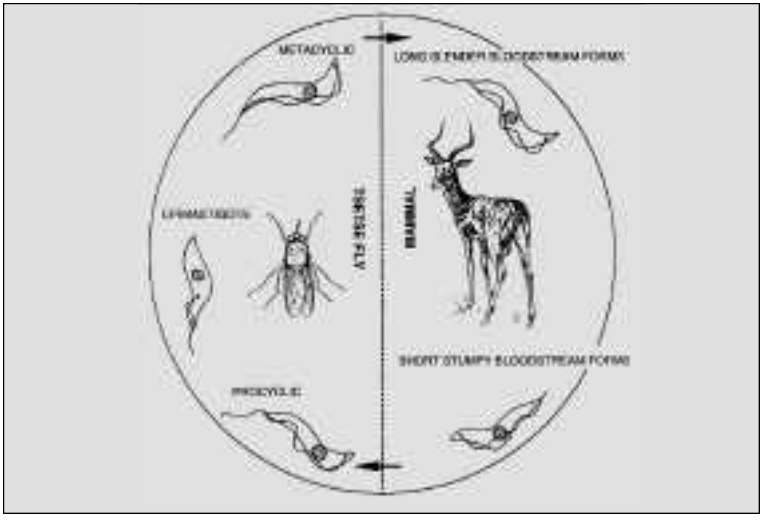


Figure 4. The life cycle of *Trypanosoma brucei.* As David Bruce realised, wild animals can act as a reservoir of the parasite. Trypanosomes are unicellular organisms; the «tail» described by Gabriel Valentin is actually a flagellum that is attached to the cell body by an undulating membrane.

Trypanosomes have to contend with very different environments in their two hosts. As extracellular parasites (bloodstream forms) which persist for months or even years in the blood and tissue fluids of the mammal, one of their main challenges is to out-manoeuvre the host immune system, which would otherwise be capable of recognising them and mounting an effective response within a few days. In addition, trypanosomes must be able to withstand digestion so that they can replicate in the tsetse fly midgut and must also follow the correct path from the gut to the mouthparts. Two distinct forms are found in the mammalian bloodstream, a proliferating long slender bloodstream form and a non-dividing short stumpy bloodstream form that is pread-apted for survival in the fly. When bloodstream forms are ingested by the fly as part of a blood meal, the stumpy form differentiates into the procyclic form. This is the establishment phase of the infection. In a second phase, known as maturation, the parasite progresses through several more rounds of differentiation, including a recently described asymmetric division (13). This gives rise to a proliferating epimastigo-te form, which in turn develops into the non-proliferating metacyclic form that is capable of infecting a new mammalian host. Epimastigote and metacyclic forms of *T. brucei* develop in the salivary glands, whe-reas the equivalent stages of *T. congolense*, a related protozoon that also causes nagana, are found in the proboscis. Trypanosomes are able to mate, but this is not an obligatory part of the life cycle. Many years ago it was proposed that the long slender bloodstream forms were male and the short stumpy forms female, but this is not the case, however enticing these stereotypes might seem. When sex takes place, it does so in the tsetse fly, but it is not yet known where this occurs, nor is it known what life cycle stages are involved.

Antigenic variation and the variant surface glycoprotein coat

Trypanolytic crises are due to the formation of antibodies in the blood. A few parasites become used or habituated to the action of these antibodies. These are the parasites which cause the relapses.

A. Massaglia, 1909

The concept of antigenic variation was formulated in the early years of trypanosome research, but more than half a century was to elapse before the molecular basis was understood. It was known that the number of parasites varied during the course of infection, but it was not until a more sensitive method of counting trypanosomes was developed by Ross and Thomson in 1910 that it became apparent that there were waves of parasitaemia in parallel to the waves of fever (14). A few years later it was shown that this also occurred during an infection initiated with a single trypanosome – an important finding since it proved that it was not due to a mixed infection with different types of parasites predominating in turn (Fig. 5).

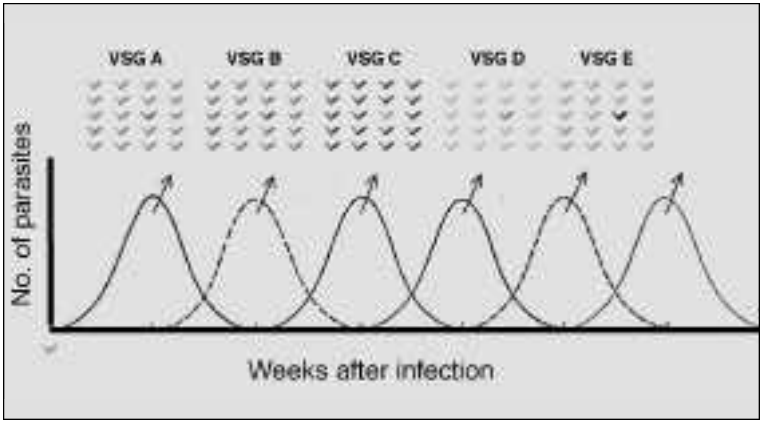


Figure 5. Antigenic variation during the course of infection. When an experimental infection is initiated with a single trypanosome, each successive wave of parasitaemia is caused by trypanosomes that are covered by a different VSG coat.

A major contribution to our understanding of antigenic variation came from the work of Keith Vickerman in 1969, when he showed that antibodies isolated during the course of a chronic infection bound to the surface of bloodstream form trypanosomes derived from earlier peaks of parasitaemia, but not with subsequent ones (15). This fitted well with the concept of immune evasion, but did not yet show how the change occurred. One theory was that trypanosomes contained a single gene for the coat protein and that a few point mutations were sufficient to alter the protein so that it could not be recognised by antibodies. The first evidence against this came when soluble coat proteins obtained from cloned trypanosomes were shown to produce distinct patterns when digested with proteolytic enzymes (16). This diversity was too great to be accounted for by minor mutations within one gene and it was proposed that each variant surface glycoprotein (VSG) was encoded by its own gene. This was confirmed by large-scale purification of VSGs in 1975 (17), with the demonstration that each had a unique amino acid composition, and the isolation of the first cDNA clones of VSG genes in 1979 (18). We now know that trypanosomes have a repertoire of several hundred genes, although not all of them may be functional.

The genetic basis of serum resistance

The success of antigenic variation lies in the ability of trypanosomes to express only one VSG at a time and to periodically switch to a new antigenic type by silencing the old gene and activating a new one. The active gene is found in an expression site that is located at a telomere. The trypanosome has different ways of recruiting a new VSG gene [reviewed in (19)]. Most of these processes involve DNA rearrangements and can also entail gene duplications and deletions. But it is the last form of antigenic variation, *in situ* activation, that is the most baffling. In this case the whole expression site is silenced and a new telomeric expression site is activated without any DNA rearrangements in either site. In fact it turns out that there may be as many as twenty potential expression sites in the parasite genome, and it most likely to

be the sub-nuclear localisation which determines whether an expression site is active or not.

In addition to the VSG gene, the expression site also contains a number of other genes, known as expression site-associated genes (ESAGs), that are coordinately transcribed [reviewed in (20)]. Some ESAGs are found in all expression sites while others only occur in asubset of them. One hypothesis is that a range of expression sites with different ESAGs might be instrumental in allowing the parasite to adapt to different hosts. One of the most exciting discoveries in recent years was the identification of a gene that can confer resistance to human serum (21). It had been known for some time that human serum-resistance was not a stable property of *T. b. rhodesiense* as it could be lost when trypanosomes were passaged in rodents and selected for in the presence of human serum. The simplest explanation was that certain types of VSG coat rendered the trypanosome resistant, but it could be shown that independent clones expressing the same VSG gene could be either resistant or sensitive. A thorough comparison of the active expression sites of sensitive and resistant strains revealed differences in their ESAGs. The resistant strain lacks several ESAGs and has an additional gene, SRA (serum resistance-associated gene), that potentially encodes a VSG-like protein. Insertion of SRA into the expression site of *T. b. brucei* rendered the parasite resistant to human serum. Since SRA is also present in the genome of *T. b. brucei*, we cannot exclude that these parasites might also become human-infectious if the gene happens to be recruited to an active expression site.

The accidental discovery of procyclins

From this point onwards this article takes a personal turn and should not be regarded as a comprehensive review of research on procyclins. My first encounter with trypanosomes took place when I joined the «Antigen Group» at the Molteno Institute in Cambridge just after completing my Ph. D. in 1983. Lucia Cardoso de Almeida and Mervyn Turner had recently discovered that VSG could occur in two forms, a

membrane form and a soluble form (4). The membrane form was shown by Mike Ferguson to contain a new class of membrane anchor that occurs widely in nature, and is particularly abundant in protozoa – the glycosylphosphatidylinositol (GPI) anchor (22). VSG could be released from the parasite surface by a mysterious enzyme, called enzyme X by the Antigen Group, and now known to be a phospholipase (23–25). Since enzyme X was known to be highly active in bloodstream forms, but absent from procyclic forms, I suggested that we use the technique of subtractive hybridisation to find genes that were specifically turned on in the bloodstream, thinking that these were likely to encode enzymes involved in VSG processing. The first step in this procedure was to make a cDNAlibrary from the «uninteresting» stage, in this case from procyclic (insect midgut) forms. Freshly made libraries can be quite unstable and it is necessary to perform an additional amplification step. This worked well for the first half, but when I came to amplify the second half a week later I found that the titre had dropped by 90%. Faced with the choice of using it or losing it, I quickly performed a differential screen and found a gene that was clearly highly expressed in procyclic forms and not in bloodstream forms. The sequence of this gene was surprising for two reasons: the encoded polypeptide appeared to be a membrane protein and it contained 22 tandem repeats of the dipeptide glutamic acid-proline (EP in the single letter code for amino acids) (26). This protein is now known as EP procyclin. At the time, the only other example of a repetitive surface protein was the circumsporozoite antigen of *Plasmodium*, and it had been proposed thattherepeats constituted an immunedecoy(27).Herewas a repetitive protein in a life cycle stage that had no contact with the mammalian immune system and was also supposed to lack a surface coat.

As so often happens in research, several groups independently converged on the same molecule from different angles. In Canada, Terry Pearson had been looking for a way to get at invariant antigens under the VSG coat and hit on the idea of raising monoclonal antibodies to the surface of procyclic forms. Ten monoclonal antibodies recognised the same antigen, but it was thought to be a carbohydrate as it could not be stained or labelled with the conventional reagents used for visualising proteins (28). Once we exchanged reagents it became clear that all

ten antibodies reacted with EP procyclin and it was because of its unusual sequence – it is highly negatively charged and contains no methionine, cysteine or aromatic amino acids (Fig. 6) – that it was biochemically invisible for so long (29).

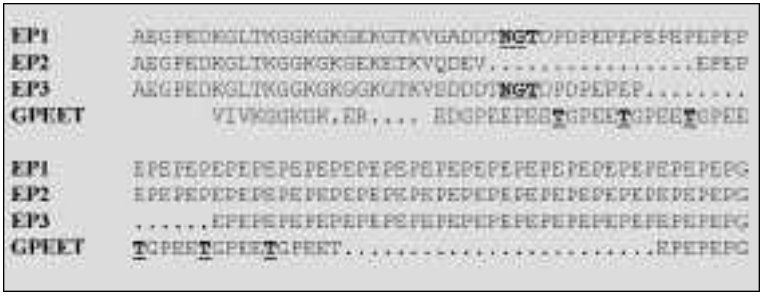


Figure 6. Primary sequences of the procyclin polypeptides aligned to maximise their similarity. Glycosylation sites in EP1 and EP3 and phosphorylation sites in GPEET are underlined. Procyclins are extremely negatively charged, not only by virtue of the glutamic acid residues in the repeats, but also because of sialic acid residues in their GPI anchors and the phosphate groups on GPEET. This, together with the predictions by nuclear magnetic resonance and molecular modelling that the repeats form extended rod-like structures, has led Terry Pearson to propose the «Spiny Norman» model in which the procyclins resemble the bristles of a hedgehog. These bristles might protect the trypanosome from digestive enzymes in the tsetse midgut and might also play a role in steering the parasite along the correct route through the fly.

At the Rockefeller University, Christine Clayton and Mike Mowatt discovered other EPprocyclins in another strain of trypanosomes (30, 31), and demonstrated that procyclins, like VSGs, are attached to the cell membrane by a GPI anchor (32). Around this time, a gene encoding a variant with a pentapeptide repeat, now known as GPEET procyclin, was discovered (33, 34). For reasons that will become clear later on, the GPEET protein was to remain invisible for a decade longer than EP.

Stage-specific expression

Saugt aber eine Zungenfliege die Trypanosomen beim Stechen eines Schlafkranken mit dem Rüssel auf, so machen die Geisseltierchen im Darm des Insektes eine eigenartige, in ihren Einzelheiten noch nicht restlos geklärte Entwicklung durch ...

G. Venzmer, Geisseln der Tropen, 1928

As Reto Brun and Margrit Schönenberger at the Swiss Tropical Institute in Basel discovered, differentiation of bloodstream to procyclic forms can also be induced efficiently in culture when the cells are shifted from 37° to 27°C and citrate and/or cis-aconitate are added to the culture medium (35). Using this system it was now possible to investigate how the parasite changed its coat and how the process was regulated. Working together with Peter Overath at the Max-Planck-Institut in Tübingen, we found that as the parasites began to differentiate they rapidly started synthesising procyclins and inserted them into the VSG coat (36). Only then was the VSG shed from the cell surface, so that the parasite was never uncoated. Depending on the strain of trypanosomes, replacing the coat can take as little as 6 hours, until each cell is finally covered by several million procyclin molecules.

In the years that followed, much of the research on procyclin centred on its stage-specific expression. What seemed at first to be a simple on-off switch, with the messenger RNA and protein being present in procyclic forms, but not bloodstream forms, turns out to be considerably more complex. One of the first surprises was the finding by Etienne Pays that procyclin genes were also transcribed in bloodstream forms (37). It now transpires that regulation occurs at several levels: transcription initiation, elongation, RNAstability and translation [reviewed in (38, 39)]. Several Ph. D. students and Masters students in Bern have made substantial contributions to our understanding of this process. Adrian Hehl was the first to show that a conserved 16mer, which was predicted to form part of a stem-loop structure in the 3' untranslated region (UTR) of all procyclin genes, was required for efficient expres-

sion, and that it was not only the sequence, but also its secondary structure that was important (40). André Furger and Nadia Schürch carried out a painstaking analysis of an entire 3' UTR in both bloodstream (41) and procyclic forms (42), finally leading to the model shown in Fig. 7.

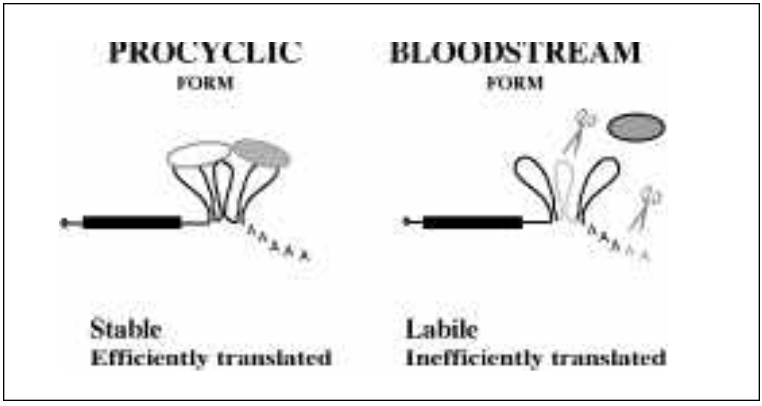


Figure 7. Schematic representation of EP1 procyclin mRNAin different conformations. The 3'UTR is predicted to form three stem-loops with the conserved 16mer at the top of the third loop. In the stable conformation (the major species in procyclic forms) one or more factors binding to positive elements in the outer stem-loops would stabilise the mRNAand ensure efficient translation. In the absence of these factors the mRNAwould be rapidly degraded by endo- or exonucleases. There might also be additional RNA-binding factors (hatched oval) which act as translational repressors. The procyclin coding region itself (black rectangle) is not intrinsically unstable, but it might contribute to the degradation of mRNAs in bloodstream forms by recruiting nucleases to the 3'UTR.

What factors interact with the procyclin 3'UTR? Despite considerable efforts in several laboratories, including ours, all attempts to identify specific RNA-binding proteins have proven unsuccessful so far. It may be that these factors are not proteins at all, but small RNAs. As has recently been shown by Matthias Liniger, there is transcription from both strands in this region of the genome (43). It is also possible that the procyclin messenger RNA is modified in vivo, and that RNA transcribed in vitro cannot interact with cellular factors. At present we are concentrating on nucleases that cleave the procyclin messenger RNA

and are also taking advantage of the trypanosome genome project to identify proteins likely to play a role in post-transcriptional regulation.

GPEET unmasked

For many years, the trypanosome coat was believed to consist solely of EPprocyclin and to be expressed by all life cycle stages except metacyclic forms (which have reacquired a VSG coat in preparation for transmission to a new mammalian host). One way to learn about the function of a gene is to produce mutants and to study their behaviour. At first sight, the procyclins are deceptively simple since, in contrast to the myriad VSG genes, there are only three types of EP gene (EP1, EP 2 and EP3) and a single GPEET gene. Stefan Ruepp, another Ph.D. student in Bern, undertook the daunting task of sequentially knocking out procyclin genes from procyclic form trypanosomes (44). The genes are organised in pairs, making it possible to delete them two at a time, but since trypanosomes are diploid four rounds of transfection were required. These went impressively smoothly for the first three pairs of genes, but when it came to knocking out the last pair, the trypanosomes always retained the GPEET gene. All attempts to delete this gene and leaving the neighbouring EP gene intact, however cunning, ended in failure. This was the first indication that there were different forms of procyclin and that they were not interchangeable. The mutant with only a single GPEET gene and no EPgenes grew absolutely normally in culture and was morphologically indistinguishable from its wild type parent. That might have been the end of the project, had it not been for the fact that one of the few tsetse laboratories world-wide is run by Reto Brun in Basel. In what was to be the first of many productive collaborations, we showed that the EPnull mutant was five to ten times less successful than its parent at establishing heavy infections in the fly midgut (44). Reintroducing a single, highly expressed copy of EP or a surface glycoprotein gene from *T. congolense* into the mutant (45) increased the number of heavy infections again.

Since no antibodies were available for GPEET, we raised polyclonal antisera against a synthetic peptide containing three pentapeptide

repeats. These antibodies bound to the surface of procyclic forms – confirming that GPEET was also part of the coat – and recognised a doublet of 20/21 kDa on immunoblots (44). One of the key questions being asked at the time concerned the relative amounts of EP and GPEET in the cell, but this could not be measured with antibodies as these could have different affinities. Fortunately, the right person to tackle this was only a few hundred metres away in Bern. Peter Bütikofer, a biochemist specialising in GPI anchors and phospholipases, labelled the wild type and mutant cell lines with anchor precursors and came back with some unexpected results. Instead of EP procyclins or the GPEET doublet that we had recently identified, by far the most abundant GPI-anchored species was a diffuse band in the range from 22-32 kDa. This was purified, sent off for protein sequencing and the answer that came back was ... GPEET! These seemingly contradictory results were resolved by computer predictions that GPEET, unusually for the extracellular domain of a surface protein, was likely to be phosphorylated on the threonine residues in the repeat. When we treated the 22-32 kDa protein with alkaline phosphatase, it now became capable of binding the anti-peptide antibodies (46). In contrast, a monoclonal antibody raised against GPEET from trypanosomes recognised the native 22-32 kDa form, but lost reactivity when the protein was dephosphorylated (47). It now appears that the 20/21 kDa form is a GPEET precursor and that phosphorylation occurs in or very close to the flagellar pocket, a specialised invagination of the plasma membrane where the flagellum emerges from the cell body. These experiments went a long way to explaining why GPEET had been overlooked for so long, but there was a further riddle to be solved: different trypanosome strains varied enormously in their relative amounts of EP and GPEET and, while some of these were stable, others tended to produce more GPEET the longer they were passaged in culture (38, 46, 48).

Differentiation revisited

Most in vitro experiments are performed with laboratory-adapted strains of trypanosomes that have been syringe passaged in rodents. These have been immensely useful, as they are easy to culture in large

numbers, but they have two serious drawbacks when it comes to questions about trypanosome biology. Firstly, long slender forms no longer differentiate to stumpy forms and for this reason such strains are termed monomorphic. Secondly, although these strains can differentiate to procyclic forms and establish midgut infections in tsetse flies, the cycle is not completed as they have also lost the capacity to produce epimastigote and metacyclic forms in the salivary glands. Pleomorphic strains have retained these important properties, but tend to lose them if they are cultured in vitro or passaged in rodents for any length of time. While at the Max-Planck Institut in Martinsried, Erik Vassella found that a recently described method for culturing trypanosomes on agarose plates enabled pleomorphic bloodstream forms to be passaged without them losing their ability to differentiate to stumpy forms (49). By optimising the method, it was possible to obtain virtually pure populations of stumpy forms that then differentiated synchronously to procyclic forms (50). Armed with this system and the full set of procyclin antibodies, one of the first things he did on (re)joining my laboratory was to re-examine how the parasites changed their coat as they differentiated from stumpy bloodstream forms to procyclic forms. As was the case for monomorphic strains (47), the pleomorphic strain turned on EP and GPEET (51). What was completely unexpected, however, was that there were differences when the newly-differentiated procyclic forms were passaged in different media. In one case, both procyclins continued to be synthesised, but in the other case GPEET disappeared within a few days while EP continued to be expressed. One of these was likely to be an artefact, but the question was which one? The answer came when tsetse flies were infected with the same pleomorphic strain. This revealed that GPEET was expressed only for the first few days and then repressed. The next step was to identify the active compound(s), something more easily said than done as there were more than twenty major differences between the two media. It turns out that GPEET continues to be expressed as long as glycerol is present in the medium and that its removal leads to the disappearance of GPEET mRNA, although the gene continues to be transcribed. Once again, it is the 3' UTR that is important for regulation, with the same element mediating the response to glycerol or oxygen tension in culture and programmed expression/repression in the tsetse fly. These experiments

represented a breakthrough for two reasons: not only did they completely reshape our ideas about an invariant procyclin coat for most of the parasite's sojourn in the fly, but they also showed that the procyclic form could sense its environment and respond by altering the composition of its coat.

Procyclins in the belly of the beast

If GPEET is turned on and off again during development, might the same hold true for the different EP isoforms? This was not a question that we could answer with the techniques that we had at our disposal, but Alvaro Acosta-Serrano and Paul Englund at Johns Hopkins University in Baltimore had recently used mass spectrometry to identify all the procyclins expressed by trypanosomes in culture (52). Once again we embarked on a collaboration and once again it proved extremely fruitful. It emerged that when pleomorphic trypanosomes differentiated in culture, they expressed their entire repertoire of procyclins for the first few hours (53). This was followed by a burst of GPEET expression, lasting a few days, to be succeeded in turn by the two glycosylated isoforms EP1 and EP3 (Fig. 7). The same pattern of expression, high GPEET followed by EP1 and EP3, was also seen in trypanosomes isolated from infected tsetse flies, but there was an important difference: fly-derived procyclins were found to lack most of the N-terminal domain upstream of the repeats. GPEET was cleaved at a specific arginine residue, while EP procyclins were cleaved at several positions around the glycosylation site (54). For the moment we can only speculate why the N-termini are removed. One possibility is that this facilitates interactions between the procyclin repeats and tsetse molecules. Alternatively, the N-terminal peptides might themselves have biological activities that influence the course of infection.

Negative results can be positive surprises

By now we are becoming quite used to the fact that trypanosomes rarely deliver the results we expect. Peter Bütikofer and I started with

a trivial question – does the 20/21 kDa GPEET precursor already have a GPI anchor? – and stumbled upon a phenomenon that might explain the anomalous behaviour of many other GPI-anchored proteins. It is well known that removing the fatty acid moieties from a GPI anchor, by treatment with phospholipases or with chemicals, generates a soluble form of the protein that no longer associates with lipid bilayers or binds to hydophobic matrices such as octyl Sepharose. When we performed these standard tests with GPEET, the molecule seemed to vanish, since we could no longer detect it with antibodies directed against the repeat. Phospholipase treatment of several other GPI-anchored molecules from parasites or mammalian cells also gave blank immunoblots – apart from the controls of course – but in one case we had the opposite result. Antibodies raised against the soluble, delipidated form of VSG reacted poorly with the intact GPI-anchored form, but showed enhanced binding after treatment with phospholipases. To cut a long story short, we have found that removal of the fatty acids from GPI-anchored molecules frequently interferes with antibodies binding to the polypeptide backbone, suggesting that the antigens have undergone major conformational changes (55). This may be one reason why the native forms of parasite surface antigens are often much more effective at protecting against infections than recombinant forms that lack an anchor. One way to surmount this problem in future might be to use (or abuse) trypanosomes as an expression system for GPI-anchored proteins.

Past and future

This seems an appropriate point to look back on the time I have spent working on parasites. When I started research on trypanosomes, most of the molecular biologists were grappling with antigenic variation and competition was stiff, to put in mildly. On the positive side, there was enormous enthusiasm and optimism that the major parasitic diseases would be under control within a few years. Today we are older and wiser. There has, however, been one important change for the better in recent years; there is much more diversity in trypanosome research and a genuine feeling of a community that works and publishes together.

What worries me is many of the younger generation of Swiss scientists perceive parasitology as a field with limited opportunities, and tend to use it as a stopover on their way to more secure/lucrative fields of research. Western society should not forget that parasites are everywhere, and that research on parasites belongs everywhere, not just in institutes bearing that name. For this reason, the Cloëtta Prize is much more than a personal honour – it is a sign that basic research in this area is still appreciated and that it deserves serious support.

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