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Role of the long slender to short stumpy transition in the life cycle of the african trypanosomes

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Abstract

It is shown using mouse models that the African trypanosomes exert a significant drain upon their host's carbohydrate (energy) resources; and that the higher the parasitemia, the greater the energy demand. It is, therefore, hypothesized that the long slender (LS) to short stumpy (SS) transition evolved, in part, to help control the parasitemia and to increase host survival time. It is also suggested that the SS population is heterogeneous. One part of the population is tsetse infective, while a second older SS population is undergoing apoptotic-like events, which leads to their cell death and their stimulation of the host's immune response. This immune stimulation by the old dying SS forms would eliminate the major LS and SS variant antigen population, and produce the chronic relapsing infection. It is concluded that the SS stages during the apoptosis-like process are acting altruistically. They give their lives to insure the long-term survival of the host, and to insure renewed growth of the minor LS variants and new infective SS forms. This process is predicted to increase the probability for the successful transmission of the trypanosomes to a new host.

Introduction: the long slender to short stumpy transition

During the life cycle of the African trypanosomes in the mammalian host, the trypanosomes undergo a developmental transition from a rapidly dividing long slender stage (LS) to a non-dividing short stumpy stage (SS). The LS stage is well adapted for growth in the mammalian host whereas the SS stage appears pre-adapted for life in the vector. The SS stage is stated not to be infective to the mammalian host and in the absence of being taken up by the vector will ultimately die. Therefore, the transition of the LS to the SS stage is not reversible. The SS stage has a relatively short half-life estimated to be between 24 to 72 hours. In the vector it proceeds to develop into the procyclic stage. The transition from LS to SS appears to be a population density dependent (or quorum sensing) event [1,2]. When the trypanosome population reaches a sufficiently high density, the trypanosomes produce a small molecular weight product (Stumpy Inducing Factor, SIF) that initiates the LS to SS differentiation [3]. Since the LS to SS transition can be induced in vitro by spent culture medium and in the absence of any host cells, the SIF is clearly not a host product [3]. It would appear that the blood stream African trypanosomes can communicate with one another through a chemical message (a trypanokine). Following SIF initiation of the LS to SS stage, one of the earliest steps in the transition is the inhibition of the cell cycle in the G1/G0 stage. This inhibition involves the cAMP pathway [4–6]. To our knowledge, the SIF has not been chemically identified but it has been suggested to be a trypanosome catabolite.

This type of stage differentiation also occurs at several other points in the African trypanosome's life cycle. Once

the SS stage has been taken up in a tsetse blood meal it will continue development to the gut procyclic stage. In this ecological niche there is rapid division that is eventually followed by a change in morphology, and the migration of an apparently non-dividing stage into the salivary glands of the vector. Once in the salivary glands, the trypanosomes attach to the salivary gland epithelium by their flagella, resume division, and form what appear to be microcolonies [7,8]. Once the number of trypanosomes reaches a sufficient density (or colony size) some of the epimastigotes undergo a morphological transition to a small, non-dividing metacyclic stage that becomes infective for the mammalian host. Similar to the LS to SS transition in the mammalian host, the epimastigote to metacyclic stage is not reversible. The metacyclic cannot change back to a dividing epimastigote form and, in the absence of inoculation into a mammalian host, will die [discussed in [9]]. The environmental signals for these differentiation events in the vector are not known but one can suggest that they are also population density dependent. Based on the physiological differences between the mammalian and vector stages, one would predict that the specific chemical inducers of these developmental transitions are different.

These types of developmental events appear to occur in many members of the family Trypanosomatidae. For example, *Trypanosoma cruzi* undergoes morphological transitions in the amastigote to trypanomastigote stage in the mammalian host and develops to the infective metacyclic stage in the hindgut of the vector. A similar change would appear to take place in the vector stages of *Leishmania* species in the transition of rapidly dividing gut stages to a non-dividing, infective metacyclic stage. The metacyclic stage ultimately dies if it is not inoculated into a mammalian host.

It would appear that many (possibly all) members of the family undergo developmental transitions that are reminiscent of biofilm and/or quorum sensing events of the prokaryotes [10–12] as well as developmental events that are observed in metazoans.

Why the LS to SS transition?

Preparation for life in the vector

It has been suggested that in the African trypanosomes there are two possible reasons for the evolution of the LS to SS transition. The first is that the LS to SS is necessary for the transition of the trypanosomes from life in their glucose-rich, highly-oxygenated blood environment into the glucose-poor, poorly-oxygenated environment of the tsetse gut. In most texts or research papers on this topic, it is suggested that it is the SS stage that is vector transmissible and there is good evidence to support this statement. The SS stage appears to be pre-adapted for life in the vector. There are changes in enzyme and mitochondrial functions suggesting preparation for survival in an environment that is low in glucose and oxygen [8]. The trypanosome populations enriched for the SS stage have been shown to be tsetse infective. However, there are also suggestions in the literature that the LS stage can be tsetse infective [discussed in [13]]. In addition, monomorphic strains can be induced to grow as procyclics in in vitro culture [14]. Is it possible that once the LS stage is locked into the G1/G0 phase of the life cycle, that these LS, pre-SS stages can in fact infect tsetse? Equally important is the question as to whether the SS population is homogenous with respect to tsetse infectivity. We hypothesize that aging SS forms eventually reach a stage in which they are no longer fly infective but in fact are programmed to die. It is, therefore, suggested that the SS population is heterogeneous: one population consists of newly formed tsetse infective stages, with a second population of old SS forms programmed to die that are not tsetse infective.

To control trypanosome growth: the trypanosome's energy demand

The second reason that has been suggested for the evolution of the LS to SS transition is to control the trypanosome parasitemia in the mammalian host and thereby prolong host survival. This would increase the time available for the successful transmission of the trypanosomes to the vector. There is considerable experimental evidence to support this hypothesis. It has been repeatedly demonstrated that monomorphic strains of the African trypanosomes are highly virulent [15]. For example one clone that we have used, the monomorphic, *T.b. gambiense*, TxTat-1 clone would kill a mouse in 5 days following the infection of a single trypanosome. The parasitemia at death was greater that 10^9 trypanosomes/ml. If one injected into the moribund mouse one ml of a concentrated glucose solution, the mouse would survive significantly longer and the parasitemia would more that double during that time period (Seed and Sechelski, unpublished). Others have reported similar results using mice and rats [16,17]. We suggest that our mice died of hypoglycemia, oxygen depletion, and acidosis as originally proposed by Von Brand [18]. A few simple calculations show that the rate of carbohydrate (glucose) utilization by the monomorphic trypanosomes at peak parasitemia is completely inconsistent with life (Figure 1). These calculations assume that 100% of the carbohydrate in the animal's diet is available to the mouse and that a terminally infected mouse has a normal appetite. It can be observed that at peak parasitemia, the trypanosomes consume/hour approximately 40% of the total mouse intake of carbohydrate/hour. This is an enormous energy drain on the host.

In contrast to the infection with a monomorphic clone, an infection of C3H mice with a pleomorphic *Trypanosoma brucei rhodesiense* clone leads to a significant increase in

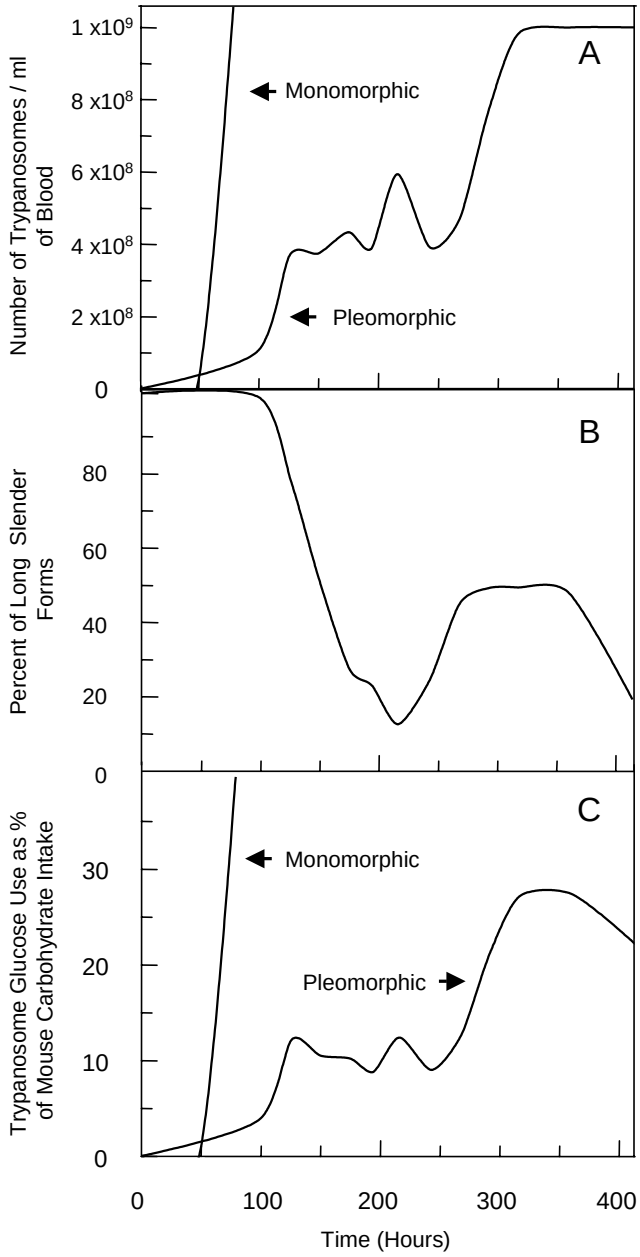

Figure 1
Susceptible C3H mice infected with monomorphic and pleomorphic trypanosomes. **Legend**: C3H mice infected with either the monomorphic TXTat-1 clone of *Trypanosoma brucei gambiense* or the pleomorphic Loutat-1 clone of *Trypanosoma brucei rhodesiense*. Panel A shows the number of trypanosomes per ml of blood. Panel B shows the percentage of the pleomorphic trypanosomes that are in the LS stage. Panel C depicts the percentage of mouse hourly carbohydrate intake (Haugh D, Technical Director Laboratory Group, Purina Mills, St. Louis Missouri, Personal Communication, 2002) that would be consumed by the circulating trypanosomes. Von Brand [18] estimated that trypanosomes use 50–100% of their dry weight in glucose each hour. For these calculations, LS trypanosomes were estimated to use 75% of their dry weight and the SS form to use half as much, or 37.5%.

survival time (approximately 20 days) and a significantly lower parasitemia (Figure 1A). The ability of the trypanosome population to switch from the LS to the SS stage lowers the parasitemia in the complete absence of any detectable host immune response (Figure 1A,1B; [19]; and others). If one calculates the carbohydrate utilization by the trypanosomes during the early peak in parasitemia of a pleomorphic population in the C3H mouse, the mouse should be able to maintain its energy balance. In these animals in this early phase of the plateau in parasitemia, the trypanosomes are calculated to consume less then 15% of their host's hourly intake of carbohydrate. It is only during the later phase with the rise in parasitemia that the consumption of host carbohydrate by the trypanosomes dramatically increases. However, even in the terminal stages the rate of glucose utilization remains under 30% of the host's total carbohydrate intake (Figure 1C). This is observed in small rodent models, in which blood glucose levels are maintained until the very terminal stages of the infection [20]. Furthermore mice infected with a pleomorphic strains show no obvious weight loss during the infection. The above calculations are based upon the assumption that the SS forms consume glucose at only 50% of the LS forms. If the SS stage has a metabolic rate for glucose similar to the LS stage the energy drain on the host would be considerably greater.

Although mice infected with a pleomorphic clone are able to survive significantly longer than animals infected with a monomorphic clone (approximately 20 days versus 5 days), the mice do not survive as long as animals that have a relapsing type infection. Immunocompetent, resistant B10BR mice have a relapsing type infection and survive an average of 38 days. We have also kept susceptible C3H mice with a normal survival time of approximately 20 days alive for an average of 71 days by periodically treating them with 2% Difluoromethylornithine (DFMO) in their drinking water in order to mimic a relapsing type infection (Seed and Sechelski, unpublished). Calculations on carbohydrate utilization by the trypanosomes during a relapsing type infection in B10BR mice are considerably reduced (Figure 2C). In B10BR mice it can be observed that the percentage of host carbohydrate utilized by the trypanosomes mimics the relapsing nature of the parasitemia (Figures 2A,2C). There are short peaks of large energy loss followed by longer periods of minimal carbohydrate utilization. It should also be noted that even at the times of the highest glucose utilization by the trypanosomes the total host carbohydrate that was lost was less than 15%. Through the LS to SS transition and by the antibody induced periods of lower parasitemia, the host would be able to replenish its carbohydrate reserves and restore homeostasis (Figure 2). This of course assumes that the host's eating habits are normal. This lowering of parasitemia would presumably allow for longer host survival.

In the above discussion, we are not trying to suggest that host carbohydrate depletion is responsible for triggering the LS to SS transition, nor do we believe that in large naturally infected mammals that energy losses are solely responsible for the pathology observed. What we are hypothesizing is that if trypanosome growth is not controlled in large animals and the parasitemia approached that found in a mouse infected with a monomorphic clone, the host would be severely energy depleted. It is suggested that one of the critical factors in the control of parasitemia is the LS to SS transition. The earlier during an infection that the LS to SS transition takes place, resulting in lower parasitemia, the less energy is lost by the host as a result of the trypanosomes' high energy demand. Although in large mammals carbohydrate catabolized by the trypanosomes is not believed to be solely responsible for the pathology, it is conceivable that, particularly in the central nervous system, local concentrations of trypanosomes could alter or damage cells by simply out-competing host cells for essential nutrients.

The calculations of glucose use by the trypanosomes in relation to the mouse intake illustrate the large energy demand that uncontrolled parasite growth places on the host. We should note that in addition to glucose the trypanosomes are also know to dramatically reduce the aromatic amino acid levels in animals with high parasitemia. It is, therefore, also conceivable that trypanosomes localized in the CNS could out compete host cells (i.e., astrocytes) for these essential amino acids as well as for glucose.

Short stumpies and the immune response

Several other points should be noted at this time. The first is that there is evidence to suggest that the LS form is more sensitive that the SS stage to antibody plus complement mediated lysis [1,21]. This would insure that the SS stages are available for transmission for a greater time period in the blood of their host. The second point is that experimental evidence has been presented demonstrating that it is the SS form that is primarily responsible for inducing the protective anti-variant specific antibody response in chronically infected mice [22]. Therefore, it would appear that the LS to SS transition is responsible for the initial non-immunological control of the peak in parasitemia, that SS forms persist in the presence of antibody for a greater period of time, and finally that it is the SS stage that is predominantly responsible for the antibody induced relapse.

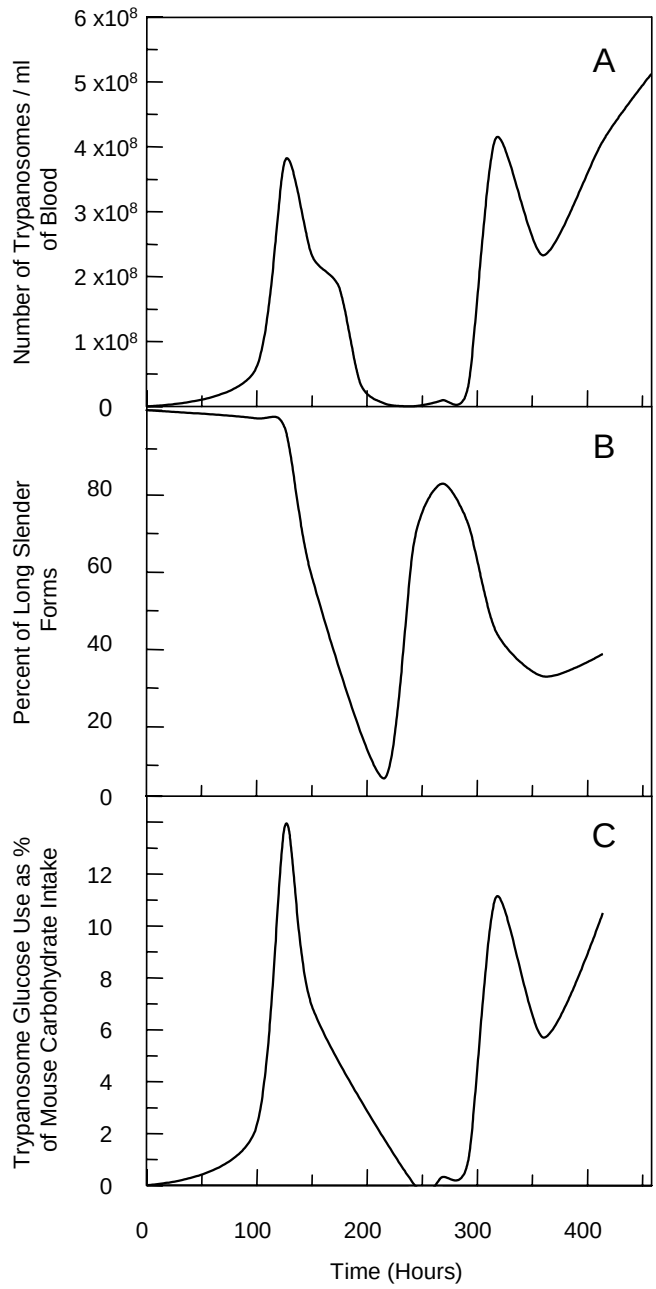

Figure 2
Resistant B10BR mice infected with pleomorphic *Trypanosoma brucei rhodesiense* **Legend**: B10BR mice infected with the pleomorphic Loutat-1 clone of *Trypanosoma brucei rhodesiense*. Panel A shows the number of trypanosomes per ml of blood. Panel B shown the percentage of these trypanosomes that are in the LS stage; at 216 hours, nearly all of the remaining parasites are the SS form. Panel C depicts the percentage of mouse hourly carbohydrate intake (Haugh D, Technical Director Laboratory Group, Purina Mills, St. Louis Missouri, (Personal Communication, 2002) that would be consumed by the circulating trypanosomes. Von Brand [18] estimated that trypanosomes use 50–100% of their dry weight in glucose each hour. For these calculations, LS trypanosomes were estimated to use 75% of their dry weight and the SS form to use half as much, or 37.5%.

Apoptosis: does it occur?

Earlier, it was asked if LS stage trypanosomes could infect tsetse as well as whether all SS forms were fly-infective. It was suggested that the SS population is not homogeneous but rather consists of young fly infective SS forms and old dying SS forms. There is little question that the SS forms in the absence of being taken up by the vector will die. It would appear that they are programmed to die during an apoptosis-like phenomenon. It seems reasonable to assume that SS forms once in their final death phase are no longer fly infective. It has been shown that the transition from LS to SS is not reversible and one can suggest that the transition from SS fly infective to SS dying is also not reversible. It is hypothesized that there is a continual transition from a LS dividing to a LS locked in the G1/G0 phase of the cell cycle to a series of morphological and biochemical stages leading to a vector infective SS form and finally to an SS form programmed to die. It is suggested that African trypanosomes from the LS form locked in the G1/G0 stage to the young SS form are all (possibly to varying degrees) fly infective. Only the rapidly dividing LS form and the old SS form are predicted not to infect tsetse.

There is current debate as to whether the SS stage undergoes apoptosis. Certainly the LS to SS transition would appear to be programmed and, in the absence of a vector, the SS stage will die. It has been demonstrated that a number of the biochemical events involved in the LS to SS transition mimic those observed in apoptosis of metazoan cells. However, at least in the *Leishmania*, the biochemical changes are not identical to metazoan apoptosis [23]. Therefore the answer to the question "Does apoptosis occur in the Trypanosomatidae?" would appear to be "no" if the definition is strictly based on the characteristics used for metazoan cells. However, it can be suggested that there is programmed cell death in the Trypanosomatidae and the process appears apoptosis-like. In our opinion, whether apoptosis or apoptosis-like events take place, the important point is that in the African trypanosomes the SS and metacyclic stages are ultimately programmed to die.

Why are short stumpies programmed to die?

At this point, one could ask why are SS forms programmed to die? Would it not be to the trypanosomes evolutionary advantage to have a continuously elevated parasitemia consisting of predominantly fly infective SS stages? A possible answer to this question can be seen by comparing the susceptible C3H mouse to the resistant B10BR animal. In the susceptible C3H mouse, the parasitemia is sufficiently high that there are large energy and nutritional demands on the host and the animal dies in a relatively shorter time period (approximately 20 days). However in the B10BR mouse, the trypanosomes have a life cycle program that ultimately leads to death of the SS population and the stimulation of the host's immune response. This leads to removal of the aging SS population as well as the LS population containing the major variant antigen type (VATs). The trypanosome population density decreases dramatically, host homeostasis is temporally restored (Figure 2), and the mouse lives approximately twice as long. Following each antibody-mediated relapse, there is renewed growth of the LS population with a new VAT. When a sufficient population density is reached, many LS trypanosomes are locked into the G1/G0 phase of the cell cycle and a new population of vector infective SS forms develops. In chronic infections this developmental cycle is periodically repeated. A chronic relapsing type infection increases host survival time and has been estimated to increase the time available for trypanosome transmission [24].

Are the trypanosomes altruistic?

In metazoan systems during differentiation, some cells act altruistically by initiating developmental events that ultimately lead to their programmed death. It can also be asked whether altruism occurs during the life cycle of members of the Trypanosomatidae. Vickerman [25] has suggested that altruism does take place. However, others debate this issue and believe that the LS to SS transition is simply part of the life cycle. It was suggested that the formation of the SS form and its death is not altruistic because there is no sacrifice of the SS form for the benefit of the trypanosome population [26].

We agree with Vickerman [25] and believe that the LS to SS transition is altruistic. If there is a transition from the dividing LS stage to vector infective forms and ultimately to a programmed dying stage, it can be argued that the dying SS forms are acting altruistically. In effect, the dying SS stage is giving its life to stimulate the host's immune response and to reduce the parasitemia. It is hypothesized that the phenomenon of antigenic variation is not simply a parasite escape mechanism but that acting synergistically with the LS to SS transition has evolved to insure parasite transmission. If Jack et al. [27] and Sendashonga & Black [22] are correct that the SS dying stage is the dominant immunogen, then the dying SS population plays a critical altruistic role in the entire process. The death of the SS stage would, therefore, insure the growth of a new LS population and a new vigorous vector infective population while increasing the total time available for the trypanosome's transmission.

Concluding comments

This paper has concentrated on one stage in the life cycle (the LS to SS transition) of the African trypanosomes. However, as noted earlier, there are other points in the life cycle of the African trypanosomes that appear similar to the LS to SS transition (i.e., the epimastigote to metacyclic,

etc.). In addition, similar morphological transitions also take place in other members of the family (i.e., *Trypanosoma cruzi* and the *Leishmania*, etc.). Although many of these developmental events appear similar, it is suspected that significant differences will be found in the environmental triggers that initiate these transitions and in the dynamics of the molecular and biochemical events involved. For example in the African trypanosomes, the LS to SS transition takes place in a free-swimming trypanosome population via a soluble trypanosome mediator. In contrast, the epimastigote to metacyclic transition occurs in a population of epimastigotes that are attached to the salivary gland epithelium and form what microscopically appear to be closely packed micro-colonies. The environmental trigger for the epimastigote to metacyclic is not currently known and, in contrast to the blood trypanosomes, may require cell-to-cell contact within the salivary gland micro-colonies.

Although we have tried to discuss a number of different topics concerning the developmental changes that occur during the life cycle of members of the Trypanosomatidae, there are a number of other questions that could also be fruitfully discussed. For example, what is the chemical identity of the SIF and where in the mammalian host does the LS to SS transition occur? There are reports that suggest that this transition is restricted to the blood of infected animals and other studies in which this transition is observed in other tissue sites [discussed in [9]]. Important questions concerning the role of the host in the transition process also exist. The observations of Black et al. [15] raise the question as to the mechanism by which a clone that was monomorphic in mice became pleomorphic when inoculated into cattle. Finally, why do pleomorphic clones not become 100% SS in immunosuppressed mice since in vitro cultures approaching 100% SS have been reported?

Our comments have hopefully generated new testable hypotheses and will stimulate scientific discussion. This paper is not intended to be a complete review of the literature. This is an exciting area of basic research and one that should receive much more attention in the future.

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References

1. Seed JR and Black SJ: **A proposed density-dependent model of long slender to short stumpy transformation in the African trypanosomes** *Journal of Parasitology* 1997, **83:**656-662.
2. Seed JR and Black SJ: **A revised arithmetic model of long slender to short stumpy transformation in the African trypanosomes** *Journal of Parasitology* 1999, **85:**850-854.
3. Reuner B, Vassella E, Yutzy B and Boshart M: **Cell density triggers slender to stumpy differentiation of *Trypanosoma brucei* blood stream forms in culture** *Molecular and Biochemical Parasitology* 1997, **90:**269-280.
4. Rolin S, Paindavoine P, Hanocq-Quertier J, Hanocq E, Claes Y, LeRay D, Overath P and Pays E: **Transient adenylate cyclase activation accompanies differentiation of *Trypanosoma brucei* from bloodstream to procyclic forms** *Molecular and Biochemical Parasitology* 1993, **61:**115-126.
5. Seebeck T, Gong K, Kunz S, Schaub R, Shalaby T and Zoraghi R: **cAMP signaling in *Trypanosoma brucei*** *International Journal for Parasitology* 2001, **31:**491-498.
6. Vassella E, Reuner B, Yutzy B and Boshart M: **Differentiation of African trypanosomes is controlled by a density sensing mechanism which signals cell cycle arrest via the cAMP pathway** *Journal of Cell Science* 1997, **110:**2661-2671.
7. Molyneux D, Lavin DR and Elice B: **A possible relationship between salivarian trypanosomes and *Glossina* labrum mechanoreceptors** *Annals of Tropical Medicine and Parasitology* 1979, **73:**287-290.
8. Vickerman K, Tetley L, Hendry KAK and Turner CMR: **Biology of African trypanosomes in the tsetse fly** *Biology of the Cell* 1988, **64:**109-119.
9. Barry JD and McCulloch R: **Antigenic variation in trypanosomes: Enhanced phenotypic variation in a eukaryotic parasite** *Advances in Parasitology* 2001, **49:**1-70.
10. Davey ME and O'Toole GA: **Microbial biofilms: from ecology to molecular genetics** *Microbiology and Molecular Biology Reviews* 2000, **64:**847-867.
11. Kolenbrander PE, Anderson RN, Blehert DS, Egland PG, Foster JS and Palmer RJ Jr: **Communication among oral bacteria** *Microbiology and Molecular Biology Reviews* 2002, **66:**486-505.
12. O'Toole G, Kaplan HB and Kolter R: **Biofilm formation as Microbial development** *Annual Review of Microbiology* 2000, **54:**49-79.
13. Hamm B, Schindler A, Mecke D and Duszenki M: **Differentiation of *Trypanosoma brucei* bloodstream trypanomastigotes from long slender to short stumpy-like forms in axenic culture** *Molecular and Biochemical Parasitology* 1990, **40:**13-22.
14. Matthews KR and Gull K: **Cycles within cycles:The interplay between differentiation and cell division in *Trypanosoma brucei.*** *Parasitology Today* 1994, **10:**473-476.
15. Black SJ, Jack RM and Morrison WI: **Host-parasite interactions which influence the virulence of *Trypanosoma* (Trypanozoon) *brucei brucei* organisms** *Acta Tropica* 1983, **40:**11-18.
16. Herbert WJ, Mucklow MG and Lennox B: **The cause of death in acute murine trypanosomiasis [abstract]** *Transactions Royal Society Tropical Medicine and Hygiene* 1975, **69:**4.
17. Sanchez G and Alderate JF: **The effect of host adrenalectomy on the physiology of *Trypanosoma rhodesiense*** *Comparative Biochemistry and Physiology* 1975, **52A:**623-626.
18. Von Brand T: **Biochemistry of Parasites** *New York, Academic Press* 1976.
19. Seed JR and Sechelski J: **Growth of pleomorphic *Trypanosoma brucei rhodesiense* in irradiated inbred mice** *Journal of Parasitology* 1988, **74:**781-789.
20. Frommel TO, Seed JR and Sechelski J: **Changes in albumen levels in blood and urine of *Microtus montanus* chronically infected with *Trypanosoma brucei gambiense*** *Journal of Parasitology* 1988, **74:**957-962.
21. McLintock LML, Turner CMR and Vickerman K: **Comparison of the effects of immune killing mechanisms on *Trypanosoma brucei* parasites of slender and stumpy morphology** *Parasite Immunology* 1993, **15:**475-480.
22. Sendashonga CN and Black SJ: **Humoral immune responses against *Trypanosoma brucei* variable surface antigens are induced by degenerating parasites** *Parasite Immunology* 1982, **4:**245-257.
23. Zangger H, Mottram JC and Fasel N: **Cell death in *Leishmania* induced by stress and differentiation: Programmed cell death or necrosis?** *Cell Death and Differentiation* 2002, **9:**1126-1139.
24. Seed JR and Sechelski J: **The role of antibody in African trypanosomiasis** *Journal of Parasitology* 1987, **73:**840-842.
25. Vickerman K: **Trypanosome sociology and antigen variation** *Parasitology* 1989, **99:**S37-S47.
26. Tyler KM: **Differentiation and Division of *Trypanosoma brucei* in the mammalian bloodsteam** *PhD thesis. University of Manchester* 1998.

27. Jack RM, Black SJ, Reed SL and Davis CE: **Indomethacin promotes differentiation of *Trypanosoma brucei*** *Infection and Immunity* 1984, **43:**445-448.