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Triatomine physiology in the context of trypanosome infection

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ABSTRACT

Triatomines are hematophagous insects that feed on the blood of vertebrates from different taxa, but can occasionally also take fluids from invertebrate hosts, including other insects. During the blood ingestion process, these insects can acquire diverse parasites that can later be transmitted to susceptible vertebrates if they complete their development inside bugs. *Trypanosoma cruzi*, the etiological agent of Chagas disease, and *Trypanosoma rangeli* are protozoan parasites transmitted by triatomines, the latter only transmitted by *Rhodnius* spp. The present work makes an extensive revision of studies evaluating triatomine-trypanosome interaction, with special focus on *Rhodnius prolixus* interacting with the two parasites. The sequences of events encompassing the development of these trypanosomes inside bugs and the consequent responses of insects to this infection, as well as many pathological effects produced by the parasites are discussed.

1. Introduction

Vincent B. Wigglesworth (1899–1994) started his career with a medical degree and a passion for insects. In 1939 he published the first edition of what became the most important book in the area: The principles of insect physiology, considered until today as a reference for researchers in the field. Due to its biological characteristics, such as easy maintenance and standardization of laboratory colonies, as well as its tolerance to harsh physical manipulation, e.g. being joined in parabiosis to other conspecifics, *Rhodnius prolixus* was chosen by Wigglesworth as a preferred model for developing experiments to discover and characterize several central physiological processes of insects. It was studying *R. prolixus*, for example, that he showed that the hormone that initiates the molting process is produced by protocerebral neurosecretory cells and that a large blood meal is necessary for hormone release (Wigglesworth, 1934). The first indications of a brain control of the acquisition of adult characteristics were also given by Wigglesworth using *R. prolixus* as experimental model (Wigglesworth, 1934). Certainly the knowledge produced by this brilliant scientist represented a critical basis to allow studying triatomine-trypanosome interaction. In this *omics* time, based on powerful computerized techniques that allow deep analyses of gigabytes of information in few hours, it is worth revisiting results produced by earlier generations where individual and creative hand work were the bases to scientific development.

2. Being an infected triatomine: sharing life with *Trypanosoma cruzi*

Trypanosoma cruzi is a protozoan parasite which infects mammals and triatomines in Latin America, while causing Chagas disease to humans. Triatomines can ingest trypomastigote forms of this parasite when they feed on the blood of infected mammals. Once inside the insect, parasites differentiate into multiplicative epimastigotes and later, in infective, non multiplicative metacyclic trypomastigotes, which are eventually released together with insect feces (Fig. 1). The development of *T. cruzi* occurs exclusively in the intestinal tract of these insects. Carlos Chagas (1909) described Chagas disease in humans and reported that triatomines were infected by this parasite. The author described the presence of round forms at the beginning of the infection and their substitution by long parasites as the infection became chronic. Dias (1934) described the development of *T. cruzi* in the triatomine *Panstrongylus megistus* (syn *Triatoma megista*). In this classical study, Dias divided bug infection into two distinct phases, the first restricted to the anterior midgut, where parasite populations entered a regression period with a prevalence of crithidial forms and, a second phase developing in the posterior midgut and rectal ampulla, in which parasites entered a multiplicative period at the former portion followed by the generation of metacyclic trypomastigotes in the rectum. Since then, several studies listed below have enhanced our comprehension of how *T. cruzi* development affects its invertebrate host.

The digestive system of insects is composed of three distinct portions that differ in contents: a foregut lined with cuticle

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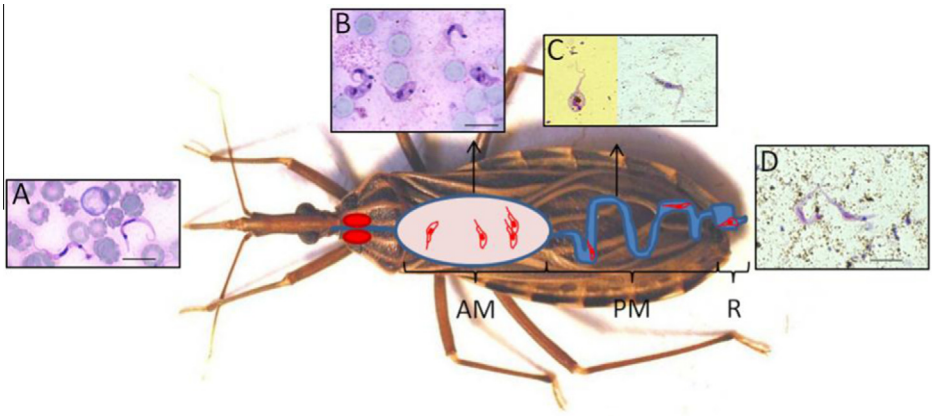

Fig. 1. Schematic representation of *Trypanosoma cruzi* development inside the triatomine. A – Blood trypomastigote; B – Trypomastigote and intermediate forms; C – Intermediate and epimastigote forms; D – Metacyclic trypomastigotes; AM – anterior midgut; PM – posterior midgut; R – rectum. Scale bars represent 10 μm.

continuous with that covering the surface of the body; a midgut whose epithelium is composed by cubical or columnar cells; and a hindgut (rectum) with a thin cuticular lining (Wigglesworth, 1950). In triatomines the midgut is divided into two portions: the anterior midgut (stomach, AM) where blood is stored and the posterior midgut (PM) where digestion occurs (Billingsley and Downe, 1985, 1988). The rectum stores the remnants of digestion and excretion processes until defecation takes place (Kollien et al., 1998).

Dias (1934) reported that the process of transformation of trypomastigotes to epimastigotes, called epimastigogenesis, happens in the PM. Nevertheless, several subsequent studies have assumed that epimastigogenesis occurs in the AM (Azambuja et al., 2005; Brener and Alvarenga, 1976; Garcia and Azambuja, 1991; Garcia and Gilliam, 1980; Garcia et al., 2010; Kollien and Schaub, 2000). This apparent contradiction, and the mostly predominant second view, had implications in our current perspective on triatomine-trypanosome interactions, since based on the latter assumption several studies only utilized epimastigotes for their experiments with the AM (Araújo et al., 2007; Azambuja et al., 2004; Castro et al., 2012; Cortez et al., 2002; Mello et al., 1996; Uehara et al., 2012). Midgut colonization by *T. cruzi* was recently evaluated and, as reported by Dias (1934), epimastigogenesis was shown to be completed in the PM of *R. prolixus* (Ferreira et al., 2016). Furthermore, the AM seems to be an inhospitable environment for *T. cruzi* since incoming trypomastigote populations are severely reduced 24 h after invading this gut portion, as shown by either fresh examination or qPCR (Dias et al., 2015; Ferreira et al., 2016). During this initial interval the remaining parasites transform into intermediate or amastigote-like forms (Ferreira et al., 2016). Intriguingly, a residual parasite population seems to survive in the AM after this initial invasion phase, even though they are only detectable by methods other than fresh examination (Dias et al., 2015; Ferreira et al., 2016). It is not clear whether these residual AM parasites display a role, if any, in the settlement of *T. cruzi* inside the vector.

The ingested blood is stored, concentrated and hemolysed in the AM of bugs (Billingsley and Downe, 1985, 1988). In this scenario bloodstream trypomastigotes enter a new microenvironment imposing dramatic changes in temperature, osmotic pressure, available nutritional resources, and the sudden interaction with bacteria and insect produced molecules. Lytic factors (Azambuja et al., 1983) and agglutinins (Mello et al., 1996; Pereira et al., 1981) seem to be differentially relevant for the establishment of different strains of *T. cruzi* (Mello et al., 1996), although this has not been fully characterized. A certain proportion of the saliva released during feeding is ingested together with the bloodmeal by the insect (Ribeiro and Francischetti, 2003; Soares et al., 2006). Therefore, it is possible that even molecules present in triatomine saliva play a role in this process. One example is trialysin, a pore-forming molecule described in *Triatoma infestans* saliva that lyses *T. cruzi* trypomastigotes (Amino et al., 2002). *In vitro* experiments have suggested that factors present in the AM of recently fed triatomines can induce parasite lysis in the beginning of infection (Ferreira et al., 2016). Protease inhibitors have been shown to be up regulated in the AM of *T. cruzi*-recently infected insects (Buarque et al., 2011; Soares et al., 2015). In addition, the arrival of *T. cruzi* also triggers the production of immunological factors (Ursic-Bedoya et al., 2008; Whitten et al., 2007) that probably affects its establishment success. As several factors can interact and influence *T. cruzi* establishment, parasites must rapidly adapt to these new conditions or be eliminated. The apparent solution found by *T. cruzi*, for which trypomastigotes are sensitive to this inhospitable environment, seems to be to quickly move as intermediate forms and reach the PM where they complete their development.

The AM of triatomines also represents the environment where most symbiotic bacteria live (Eichler and Schaub, 2002). Bacteria numbers in the AM can increase approximately 10,000 times 48 h after blood ingestion (Azambuja et al., 2004), staying at high levels up to seven days later (Eichler and Schaub, 2002). Nevertheless, the drastic reduction of *T. cruzi* numbers 24 h after infection does not seem to be related with symbiotic bacteria, since it has been shown that the parasite killing at that period is similar in *R. prolixus* in either presence or absence of *Rhodococcus rhodnii* (Dias et al., 2015). It is worth mentioning that although not being responsible for *T. cruzi* number reduction, gut microbiota does interact with the parasites. In fact, a kazal-type protease inhibitor (*rRpTI*) was recently shown to be up regulated in the AM of *T. cruzi*-recently infected *R. prolixus* (Soares et al., 2015). Insects that were knockdown for *rRpTI* showed reduced parasite and increased bacterial abundance in their AM, which seems to suggest that this molecule can affect *T. cruzi*-bacteria interactions during the first hours of infection (Soares et al., 2015). On the other hand, the presence of *T. cruzi* induces a decrease in bacterial populations present in the AM of *R. prolixus* during the first days of infection (Castro et al., 2012). In this case, the reduction in bacterial load associated with a rise in *T. cruzi* populations was related to increases in antibacterial activity, including that of the phenoloxidase pathway (Castro et al., 2012). In chronic infections bacteria and parasites are physically apart, bacteria developing mainly in the AM and *T. cruzi* fundamentally in the PM and rectum (Eichler and Schaub, 2002).

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