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MOLECULAR BIOLOGY OF THE INTERACTION *TRYPANOSOMA CRUZI*/ INVERTEBRATE HOST

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Trypanosoma cruzi, the causative agent of Chagas' disease, requires triatomine insects for completion of part of its life cycle in nature. The morphogenetic processes which take place in the insect digestive tract, i.e., differentiation of blood trypomastigotes to epimastigotes and/or spheromastigotes, epimastigote multiplication, and the transformation of epimastigotes to metacyclic trypomastigotes (the form naturally infective to vertebrate hosts) are crucial steps in maintaining the *T. cruzi* life cycle in nature.

One of the major gaps in *T. cruzi* biology concerns the interaction of the parasite with its invertebrate host. Apparently this interaction is dependent on numerous parameters related to the insect species and/or parasite strain (Brener, 1973).

The purpose of this paper is to discuss the present knowledge of the molecular biology and to raise some questions that remain unanswered on the interaction of *T. cruzi* and its invertebrate host.

Trypanosoma cruzi life cycle in the invertebrate host

Although the ability of *T. cruzi* to infect and develop in triatomines seems to be an atractive model for investigating the relationship of vectors and pathogenic organisms, only a few extensive studies have been carried out in this specific area of study. The basic findings on the development of *T. cruzi* in the insect have long been described (Chagas, 1909). However, the first work to treat the parasite cycle in the triatomine comprehensively was that of Dias (1934). Recently, Alvarenga (1979) described the life cycle of *T. cruzi* in more detail. The authors showed that the cycle begins with the ingestion of circulating trypomastigotes in the blood meal, which transform to epimastigotes and/or spheromastigotes, the epimastigote forms multiply and differentiate to metacyclic trypomastigotes as the blood moves along the gut, eventually accumulating the rectum, from where they are expelled. Other aspects of the life cycle of *T. cruzi* in the invertebrate hosts that have been investigated are different susceptibility of individual triatomine species (Ryckman, 1965; Urdaneta-Morales, 1973; Miles et al., 1975; Zeledón, 1976; Perlawagora-Szumlewicz & Muller, 1979), and metacyclogenesis of different *T. cruzi* strains and/or clones in triatomine insects (Urdaneta-Morales & Rueda, 1977; Garcia & Dvorak, 1982).

These findings call our attention to an unanswered problem: can the invertebrate host provide a selective advantage for the development of an intrastrain variant of *T. cruzi* over other variants, and can different strains or clones of *T. cruzi* coexist without alterations in their behaviour in the same triatomine species, or will they necessarily complete?

Kinetics of *Trypanosoma cruzi* transformation and development in the digestive tract of triatomines

In our laboratory we have studied some of the above mentioned aspects of *T. cruzi* life cycle. We have observed the differentiation of trypomastigotes to spheromastigotes and/or epimastigotes, the epimastigote multiplication, and metacyclogenesis of different *T. cruzi* strains and clones. Fig. 1 illustrates the transformation of trypomastigotes which occurs mainly in the insect's crop. We observed that the rate of transformation is variable and it seems to depend on the nature of the strains or clones of *T. cruzi*. Furthermore, we have examined epimastigote multiplication and metacyclogenesis of the above mentioned strains and clones in *Rhodnius prolixus*. Fig. 2 shows that the rate of growth of these parasites is also very variable, and it is related to the first *T. cruzi* differentiation, i.e., the strains of *T. cruzi* which transform rapidly to epimastigotes and/or spheromastigotes have better conditions to multiply and establish the infection as well as to produce metacyclic trypomastigotes (Table I).

As a whole, these observations showed that the differentiation of trypomastigotes, the multiplication of epimastigotes, and the metacyclogenesis are interdependent phenomena in relation to the insect species. Although the inocula of *T. cruzi* used in these experiments to infect *R. prolixus* were undoubtedly higher than those occurring in nature, it seems that the variations found in the rate of infection depend on the parasite strain or clone itself rather than on the number of parasites used.

On the other hand, it should be emphasized that the growth and differentiation of the WA-250/1 and Esmereldo/2 clones seems to be dependent on the insect species used as vector. In *R. prolixus* these two clones presented low levels of differentiation and development (Fig. 1 and Fig. 2). However, we have

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TABLE I

Percentage of a epimastigotes and metacyclic trypomastigotes from different strains and/or clones of *Trypanosoma cruzi* from post-molting adults of *Rhodnius prolixus* at 30 days after feeding. The insects were infected as fifth-instar with 5 x 10^4 trypomastigotes/ml of blood as described by Garcia et al. (1984a)

T. cruzi strains and/or clones	Percentage	
	Epimastigotes	Metacyclic trypomastigotes
W	45	55
Costa Rica 510	59	41
Y	75	25
WA-250/1	85	15
Esmereldo/2	90	10

shown, by examining the development of these two clones in *Dipetalogaster maximus*, that the clone WA-250/1 underwent epimastigote to metacyclic trypomastigote morphogenesis in the 30 day course of the experiments. The clone Esmereldo/2 did not undergo transformation to metacyclic trypomastigotes, even though both presented high levels of infection in the insect (Garcia & Dvorak, 1982). Since the WA-250/1 and Esmereldo/2 clones of *T. cruzi* are biochemically typed as zymodeme I and zymodeme II, respectively (Dvorak, Hartman & Miles, 1980), and their kDNA schizodeme profiles are different using EcoRI restriction endonuclease (Morel, Gonçalves & Dvorak, personnal information), we conceive, with some confidence, that the behaviour of *T. cruzi* in its invertebrate host may depend on the biochemical characteristics of the strains or clones of the parasites in question.

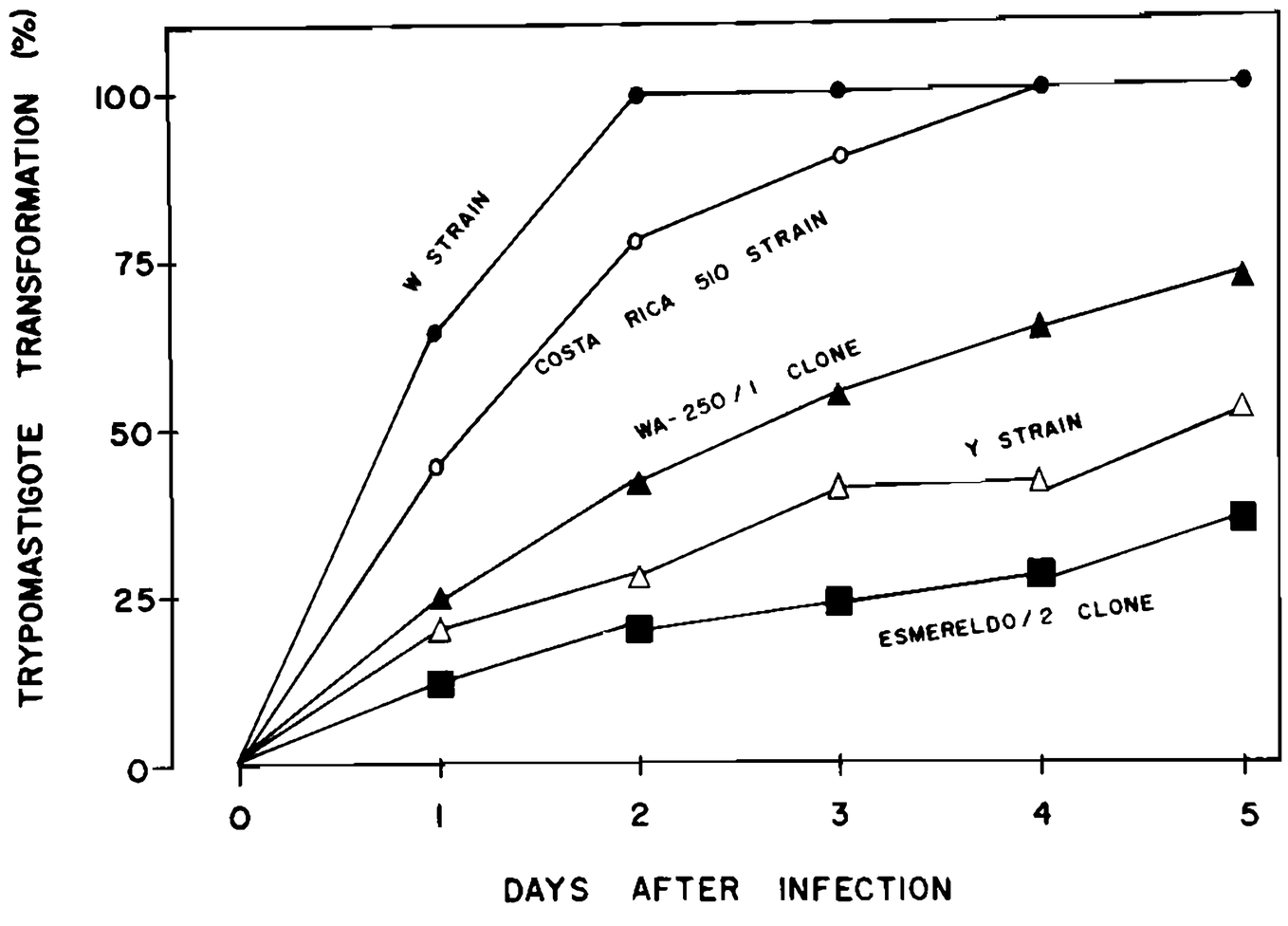

Fig. 1: percentual transformation of trypomastigotes from different strains and/or clones of *Trypanosoma cruzi* in the crop of fifth-instar larvae of *Rhodnius prolixus*. The insects were infected with 5 x 10^6 parasites/ml of blood through the method described by Garcia et al. (1984a) and dissected at 1, 2, 3, 4 and 5 days after feeding.

The foregoing findings led us to speculate that the insect itself can be considered one of the main barriers against the establishment of *T. cruzi* infection in the vector. An early hypothesis advanced to explain invertebrate host-parasite specificity would be that the digestive process of the insect could determine the vectoring ability. It must not be forgotten, for example, that the lytic factor present in the crop is active where trypomastigote transformation occurs. This factor is important since by lysing the erythrocyte membranes haemoglobin is released for digestion by proteolytic enzymes (Azambuja, Guimarães & Garcia, 1983). This factor may also lyse *T. cruzi* membranes and be an important selective agent for determining the establishment and intensity of infection by different *T. cruzi* strains. On the other hand, it is known that blood meal components are attacked by hydrolytic enzymes in the intestine (Garcia, 1985) which is the place where epimastigotes multiply intensively. It is therefore possible that the digestive enzymes besides liberating nutrients for the development of *T. cruzi* could also be considered as aggressive factors destroying the parasites. These hypothesis have been taken into account in our laboratory and remain as an attractive model for explaining the invertebrate host-*T. cruzi* specificity.

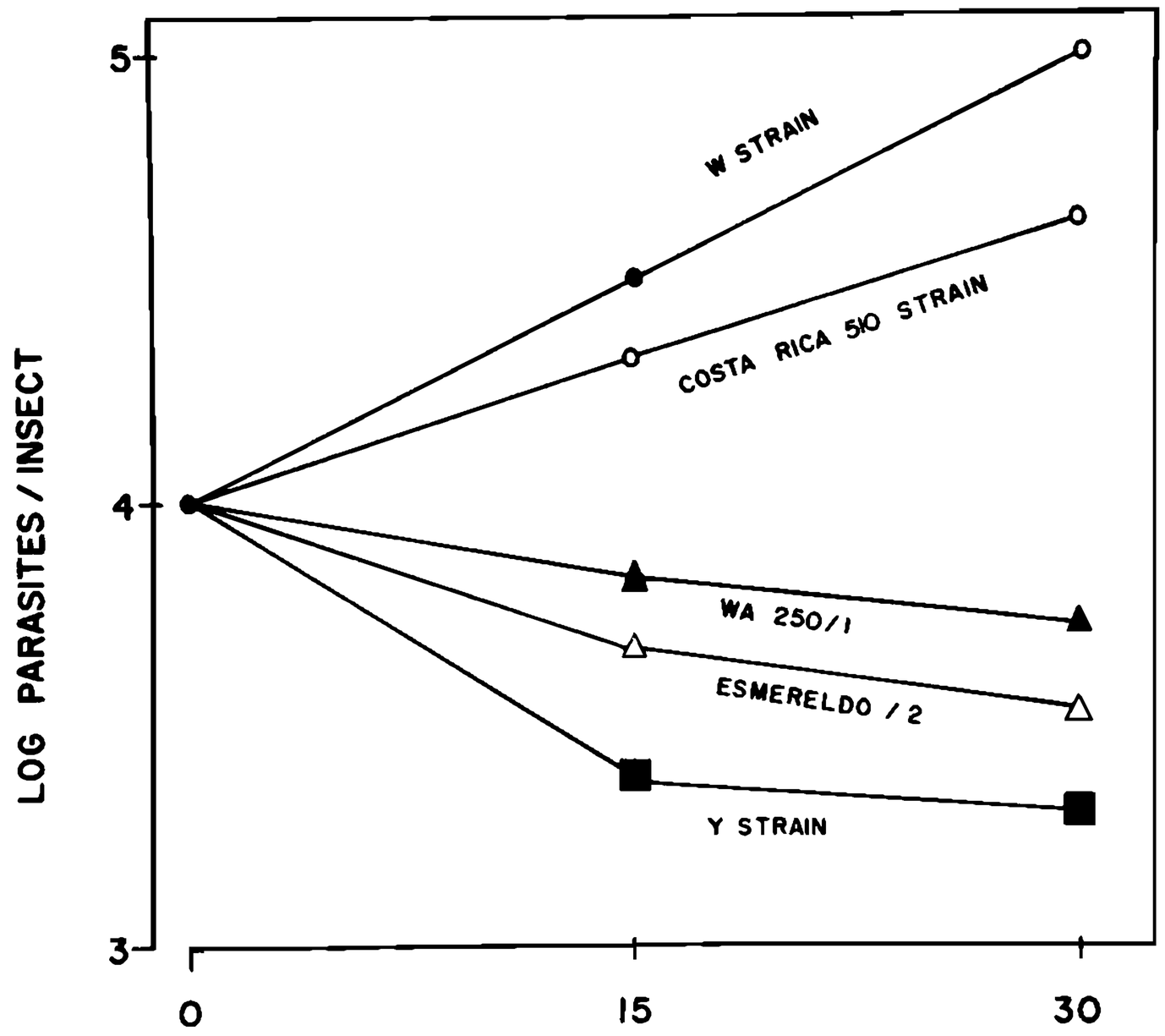

Fig. 2: development of different strains and/or clones of *Trypanosoma cruzi* in the gut of fifth-instar larvae of *Rhodnius prolixus* at 15 and 30 days after feeding. The insects were infected with 5 x 10^4 trypomastigotes/ml of blood as described by Garcia et al. (1984a).

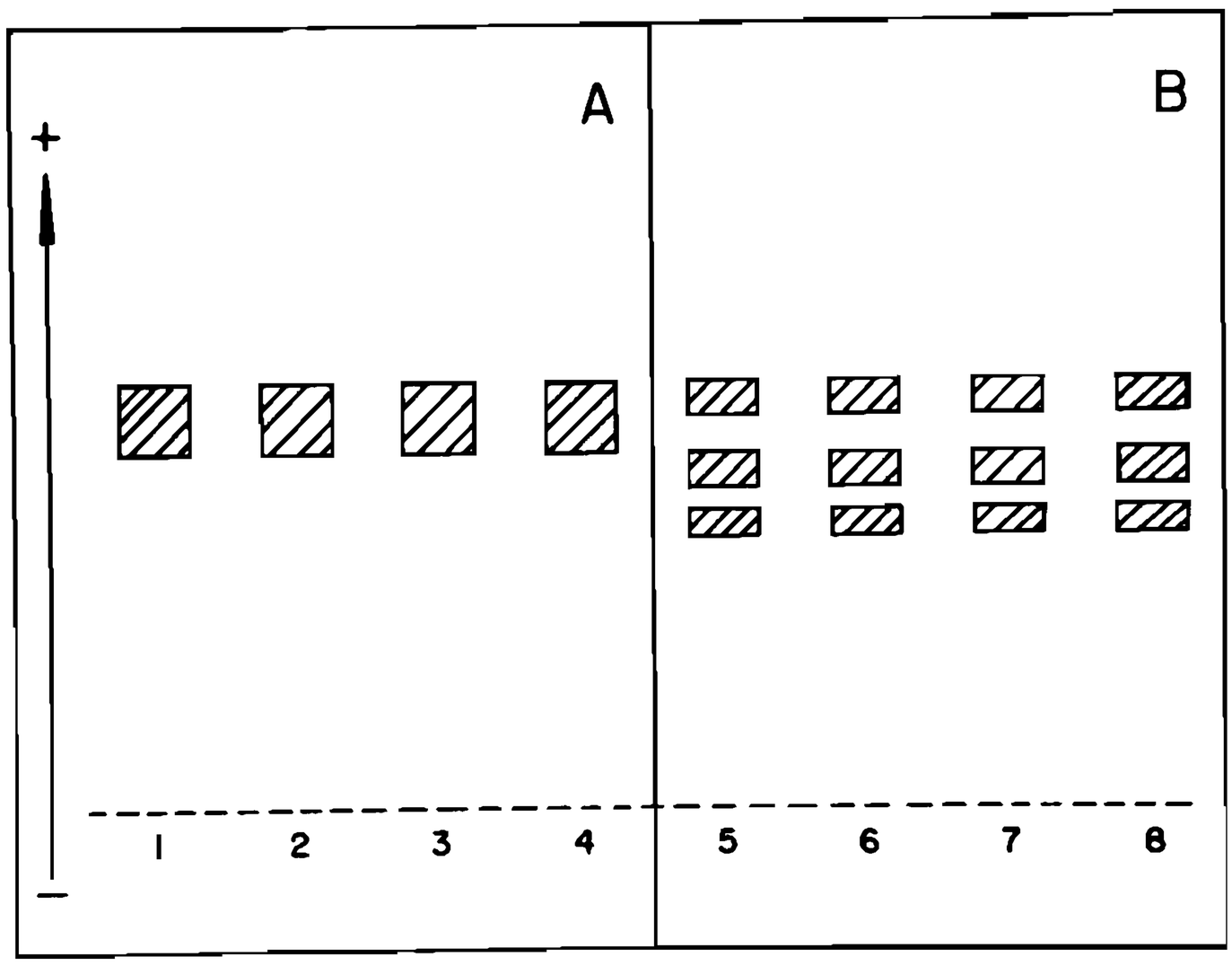

Fig. 3: diagrammatic representation of agarose electroforetic profiles of (A) glucose phosphate isomerase (GPI, E.C.5.3.1.9.) and (B) phosphoglucomutase (PGM, E.C.2.7.5.1.) isozymes of *Trypanosoma cruzi*. The parasites were isolated from *Panstrongylus megistus* (1-5); *Triatoma infestans* (2-6); *Triatoma brasiliensis* (3-7) and *Rhodnius prolixus* (4-8).

Passage of a *T. cruzi* strain through different vectors

A question that arises is whether different triatomines would have the ability to modify the genotype and/or phenotype of a biochemically well defined *T. cruzi* strain. To answer this question, we have used zymodeme (Miles et al., 1978; Miles, 1979) and schizodeme (Morel et al., 1980) techniques as biochemical markers to investigate the passage of the W strain of *T. cruzi* through four different insect species: *R. prolixus, T. infestans, T. brasiliensis* and *P. megistus*. Fig. 3 and Fig. 4 show the zymodeme and schizodeme profiles, respectively, of parasites isolated from the urine of chronically infected invertebrate hosts. These results indicated a high unsuspected degree of homogeneity of all the parasites isolated from the insects, and that there were no modification in the zymodeme and schizodeme levels by the passage of W strain through these different vectors (Garcia et al., 1984b, c). Furthermore, these experiments did not alter any of the following biological parameters of the W strain that was isolated from the guinea pig upon which

the insects were fed: (a) convertion ratio of epimastigotes to metacyclic trypomastigotes; (b) parasitemia curve in mice inoculated with insect urine metacyclic trypomastigotes; (c) doubling time in LIT medium and in the invertebrate hosts.

Finally, we have used monoclonal antibodies to detected the surface antigens of metacyclic trypomastigotes present in the urine from these four vectors. In all cases, the monoclonal antibodies showed similarity of surface antigens of metacyclic trypomastigotes, i.e., they were unable to discriminate the strains obtained from the insects assayed (Garcia et al., 1984c).

Would the insect be able to select subpopulations of *T. cruzi* within heterogeneous populations of parasites?

It is known that *T. cruzi* strains may be composed of heterogeneous mixtures of morphologically indistinguishable organisms (Miles et al., 1977; 1978). It would therefore be possible that the insect could select subpopulations from a natural population. Preliminary experiments from our laboratory suggest that this hypothesis may be true. These studies, however, need to be confirmed. Furthermore, we are concomitantly studying the possibility that different *T. cruzi* strains can simultaneously infect the insect, and whether mixed infection or reinfection in the invertebrate hosts occur.

Fig. 4: EcoRI schizodeme analyses of *Trypanosoma cruzi* isolated of *Panstrongylus megistus* (A), *Triatoma infestans* (B), *Triatoma brasiliensis* (C), and *Rhodnius prolixus* (D) fed on a naturally infected guinea pig.

Conclusion and Prospects

In this paper the molecular aspects of the life cycle of *T. cruzi* in its vector was discussed. It should be emphasized that the literature on this respect is scarce and most of it deals with the description of parasitological aspects of this cycle. Firstly, we considered the differentiation of trypomastigotes to epimastigotes and/or spheromastigotes which occurs soon after the blood meal ingestion. Secondly, we discussed the multiplication of epimastigotes and their transformation to metacyclic trypomastigotes using different *T. cruzi* strains and/or clones. These findings were related to the studies using biochemical markers such as zymodemes and schizodemes as well as monoclonal antibodies against surface antigens to characterize the parasites. We concluded that the use of such biochemical parameters will help us in the elucidation of the factors imposed by the insect on the parasite or vice-versa.

Recent *in vitro* and *in vivo* studies on *T. cruzi* gene expression (Goldenberg et al., present Symposium) and the use of kDNA sequences in the detection and characterization by dot-blot hybridization of *T. cruzi* strains (Morel et al., present Symposium) are promising new tools to clarify the many questions that remain to be answered in the invertebrate host-*T. cruzi* relationship. We are looking forward to a busy future.

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