

An Observation of the Transitional Forms between Trypomastigote and Amastigote of *Trypanosoma cruzi* by Scanning Electronmicroscopy

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Abstract: The transformation process between the trypomastigote and the amastigote was studied by Scanning Electronmicroscopy (SEM). The exclusive process from the trypomastigote to the amastigote was accomplished by incubating trypomastigotes in LIT medium while that from the amastigote to the trypomastigote, by rupturing parasitized fibroblasts after removal of free parasites from the fibroblast cultures infected with *T. cruzi*. Principally, the former process was initiated by twisting and folding of the parasite body, and the latter, by elongation of the body.

Key words: *Trypanosoma cruzi*, Trypomastigote, Scanning Electronmicroscopy, Transformation

INTRODUCTION

In nature, *Trypanosoma cruzi* amastigotes are found only within mammalian cells, while trypomastigotes are extracellular. Many works have been done to understand the transformation mechanism although real regulatory factors have not yet been cleared. Morphological studies on the transformation process advanced rapidly by employing the tissue culture technique and by developing various culture media. Before the naming of the transitional forms (Hoare and Wallage, 1966; Brack, 1968), the morphological change in the life cycle of *T. cruzi* had been studied intensively. Thereafter, new morphological aspects of intracellular development from trypomastigotes to amastigotes, and vice versa, were demonstrated by optical microscopy (Behbehani, 1973; Dvorak and Hyde, 1973) and by transmission electron microscopy (Inoki *et al.*, 1971; Sooksri and Inoki, 1972; Pan, 1978) Although these works revealed various information of the transitional stages between the amastigote and the trypomastigote, the external view, mainly the relationship between the flagellum and the body was obscure. Andrews *et al.* (1987) clearly showed this aspect of the transformation

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Received for Publication, May 22, 1991.

Contribution No. 2532 from the Institute of Tropical Medicine, Nagasaki University.

from trypomastigotes to amastigotes using SEM. In the present work, we observed both courses from the trypomastigote to the amastigote and from the amastigote to the trypomastigote by SEM.

MATERIALS AND METHODS

Parasite:

The H-23 clone derived from the Tulahuen strain of *T. cruzi* was used (Kanbara *et al.*, 1988).

Transitional forms from trypomastigotes to amastigotes (Sample-1)

Trypomastigotes were isolated through a CM-cellulose column (Kanbara and Nakabayashi, 1985) from overlaid media on mouse fibroblast monolayer cultures infected with *T. cruzi*. Trypomastigotes were sedimented by centrifugation at 1,800 g for 10 min, suspended in a modified LIT medium at a concentration of 2×10^6 /ml, and incubated at 25°C. After incubation for 24 h and 48 h, trypanosomes were collected, washed once in PBS (pH 7.3) and fixed in 2% glutaraldehyde in PBS (pH 7.3) for 2 h at 4°C. These specimens consisted of only transitional forms from trypomastigotes to amastigotes. Mouse fibroblasts used in the present work were derived from the skin of ICR newborn mice and were established in culture.

Transitional forms from amastigotes to trypomastigotes (Sample-2).

Mouse fibroblasts were infected with H-23 trypanosomes maintained in the modified LIT medium, and were incubated at 37°C in 5% CO₂-atmosphere. Culture media (MEM supplemented with 10% newborn bovine serum) were changed every 2 to 3 days. When more than 80% of fibroblasts were observed to contain parasites, they were gently washed thrice with PBS (pH 7.4) to remove free parasites from the fibroblasts. Then, 20 µg/ml of trypsin solution (Sigma, Type III) in PBS (pH 7.4) containing 0.02% EDTA was added to separate the fibroblasts from the bottoms of culture dishes. Separated cells were washed once with PBS, suspended again in PBS and homogenized at 1,000 rpm by a Teflon-homogenizer in ice until many free parasites became visible by microscopy. The specimens were centrifuged, washed once with PBS, fixed and subjected to SEM.

Scanning Electronmicroscopy (SEM)

Fixed specimens were washed twice in PBS (pH 7.3) for 1 h and suspended in PBS. Several drops of suspension were put on a piece of 0.1%-poly-L-lysine-coated round aluminum foil which was placed at the bottom of a test tube. The sample was centrifuged at 500 rpm for 5 min. The adhered specimens on the aluminum foil were dehydrated in graded ethanol and critically point-dried from liquid CO₂ with the use of isoamylacetate at the transition fluid. The specimens were coated with gold-palladium in JEC-1100 Ion Sputtering Device before being examined in a JEOL 100 CX scanning electronmicroscope.

RESULTS AND DISCUSSION

When trypomastigotes from fibroblast cultures were transferred to a modified LIT

medium, they transformed into amastigotes sooner or later but not synchronously. The sample, therefore, contained various transitional stages from the trypomastigote to the amastigote. As Andrew *et al.* (1987) pointed out, trypomastigotes twisted and folded themselves, consequently shortening their bodies (Fig. 1). These folded forms were often seen in Sample-1 but the transitional forms from the folded trypomastigotes to sphaeromastigotes or amastigotes were rarely seen. This indicates that the process might involve the rapid biochemical dissolution of flagella. It is uncertain whether shortening a body always starts from its twist. Kanbara *et al.* (1990) observed that the transformation from the trypomastigote to the amastigote was accelerated by the condition of low pH. Fig. 2 shows forms after 5 h incubation of trypomastigotes at pH 5.0 in which many short and stumpy trypomastigotes were seen. Since flagella were not stained clearly in this figure, transforma-

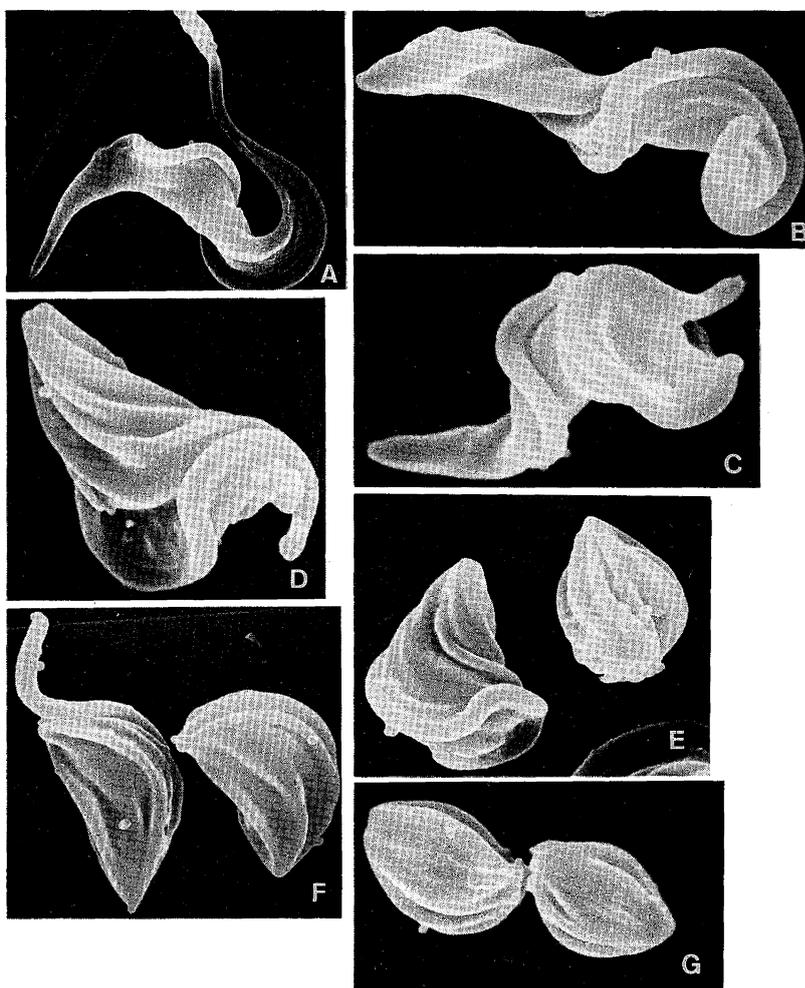


Fig. 1. Transformation process from trypomastigote to amastigote in LIT medium. Typical trypomastigotes (A, B) initially twisted their bodies which were shortened by folding themselves (C–E) and finally transformed to amastigotes (F, G).

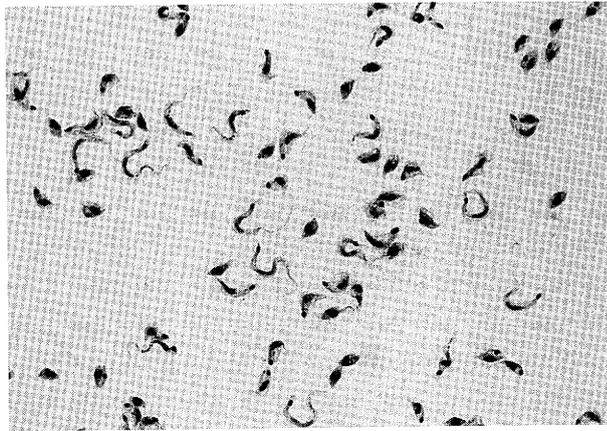


Fig. 2. Transitional forms (arrows) from trypomastigotes to amastigotes in a low-pH medium.

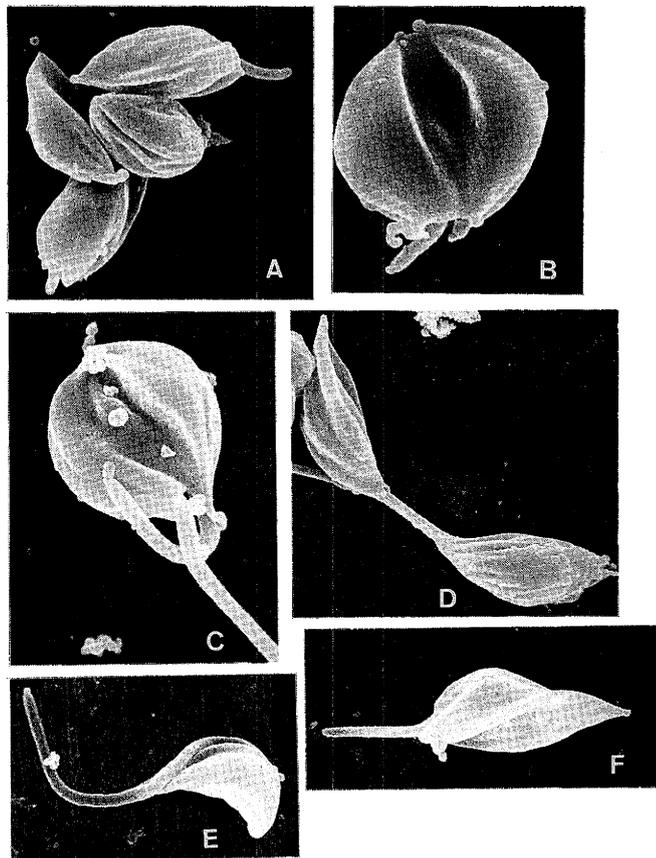


Fig. 3. Transformation process from amastigote to trypomastigote in fibroblast cells. Amastigotes (A, B) generally possessed tiny flagella. Cell division was found at the stage of the amastigote (B), and also of the transitional forms (C, D). Principally, transformation involved the elongation of the body and the flagellum (E, F).

tion seemed to be initiated simply by rounding the bodies. A resulting sphaeromastigote or amastigote generally shows a slightly long dome-like shape (Fig. 1F), one edge is tapering and the other, blunt. Even on amastigotes in this sample, flagella along the bodies were still visible.

The transformation from amastigote to trypomastigote in Sample-2 revealed that the flagellar extension and the elongation of the body concurrently but independently progressed (Fig. 3). The elongation of the body involved a slight twist. Amastigotes derived from infected fibroblasts were fusiform and rather longer than those in Sample-1 and, almost exclusively, had tiny flagella. Since only heavily parasitized cells were destroyed by Teflon-homogenization, some differences were expected between the amastigotes released from these fibroblasts and those released from slightly parasitized cells which were not observed in the present work. The course of cell division and that of transformation are thought to be independent of each other and not to be synchronous. Therefore, dividing forms at different stages were observed (Figs. 3B, C, D). Furthermore, in the transformation process, the body elongation and the flagellar extension did not seem to progress synchronously. Thus, transitional forms exhibited wide variation which was thought to have led to the idea of the two processes of transformation (Behbehani, 1973). In Fig. 4, we illustrate the simplified process of the cycle between the amastigote and the trypomastigote. SEM clearly shows the relationship between the body and the flagellum.

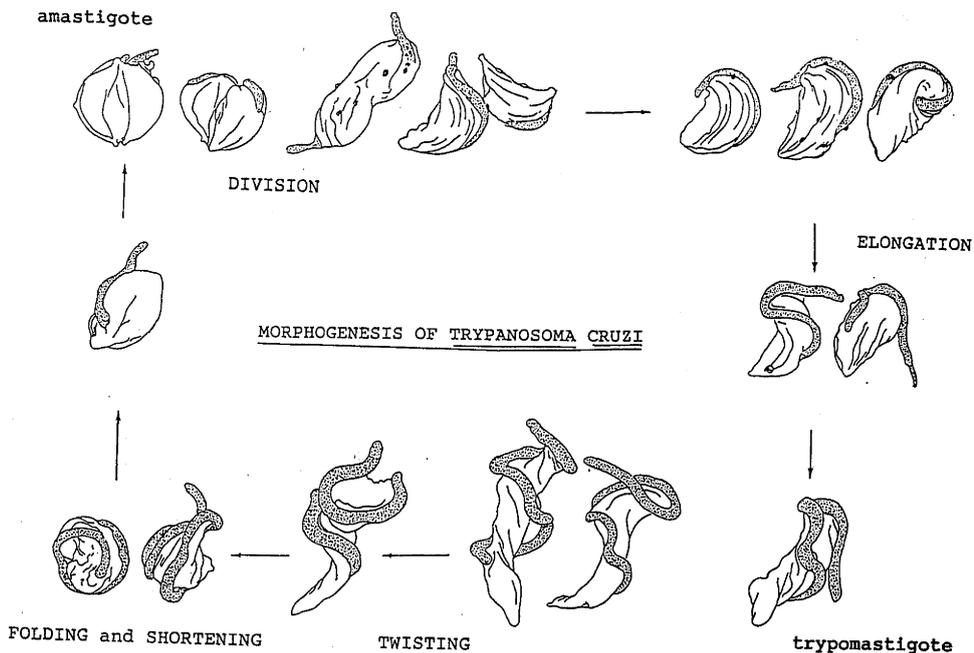


Fig. 4. Illustration of the transformation process between the trypomastigote and the amastigote.

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