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MiniReview

Endosymbiosis in protozoa of the Trypanosomatidae family

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Abstract

A small number of trypanosomatids present bacterium endosymbionts in the cytoplasm, which divide synchronously with the host cell. *Crithidia oncopleti*, *Crithidia deanei*, *Crithidia desouzai*, *Blastocrithidia culicis* and *Herpetomonas roitmani* are the best characterized species. The endosymbiont is surrounded by two membranes separated from each other by an electron-lucent space. The presence of the endosymbiont led to the appearance of morphological changes which include the lack of the paraflagellar rod associated to the axoneme, the morphology of the kinetoplast and the association of the sub-pellicular microtubules with portions of the protozoan plasma membrane. Aposymbiotic strains could be obtained by antibiotic treatment, opening the possibility to make comparative analysis of endosymbiont-containing an endosymbiont-free populations of the same species. It is clear that metabolic cycles are established between the prokaryiont and the host cell. The results obtained show that endosymbiont-containing species of trypanosomatids constitute an excellent model to study basic processes on the endosymbiont–host cell relationship and the origin of new organelles.

Keywords: Endosymbiont; Biochemistry; Phylogeny; Trypanosomatid; Ultrastructure

1. Introduction

Protozoa of the Trypanosomatidae family comprise a large number of species, some of which are agents of important illnesses, such as leishmaniasis, Chagas' disease and African trypanosomiasis, affecting man and animals of economic interest. In addition, trypanosomatids have emerged as important models for the study of basic biological processes, including RNA editing and *trans*-splicing, organization of extranuclear DNA, antigenic variation, etc. A relatively small number of trypanosomatids have been described containing bacterium symbionts in the cytoplasm, known as endosymbionts, first described as diplosomes by Novey et al. [1] in *Blastocrithidia culicis*. In 1957, Newton and Horne [2] reported the presence of similar structures, designated as bipolar bodies in *Crithidia oncopelti*, a trypanosomatid isolated in 1926 by Noguchi and Tilden [3] from the digestive tract of the hemiptera *Oncopeltus fasciatus*. Using cesium chloride gradient Marmur et al. [4] showed that these bodies contained DNA similar to that found in bacteria. This finding was supported by the observation that the diplosomes disappeared from cells treated with chloramphenicol [5]. Later on, other endosymbiont-containing species were isolated from insects in Brazil (Table 1).

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Table 1
Trypanosomatid species which harbor endosymbionts

Insect species	Source	Culture	Cured strain	Reference
Blastocrithidia culicis	*Triatoma infestans*	+	+	[1]
Crithidia deanei	*Zelus leucogrammus*	+	+	[25]
Crithidia desouzai	*Ornidia obesa*	+	−	Fiorini et al., 1989; [9]
Crithidia oncopelti	*Oncopeltus fasciatus*	+	+	[2]
Herpetomonas roitmani	*Ornidia obesa*	+	+	Faria e Silva et al., 1989; [17]

2. Morphology

The examination of Giemsa-stained trypanosomatids shows that symbionts are usually localized close to the host nucleus, with a length of 1.3–2.3 μm and a width of 0.3–1.0 μm. Each cell harbors one or two endosymbionts which divide synchronously with the host cell. Transmission electron microscopy of thin sections shows the endosymbiont surrounded by two membranes separated from each other by an electron lucent space of about 28 nm (Figs. 1–3). The thickness of the inner and the outer membranes is about 7.0 and 8.5 nm, respectively. Close contacts between the two membranes are observed at some points [6–8]. The examination of freeze-fracture replicas shows that there are differences in the density of intramembranous particles (IMPs), which may correspond to membrane integral proteins, between the outer and the inner membranes (Fig. 4). The IMPs are more closely packed on the E fracture face of the outer membrane than on the P face. In contrast, the density of particles was higher on the P than on the E fracture face of the inner membrane. This pattern of particles distribution found for *Blastocrithidia culicis* and *Crithidia desouzai* [8,9] is similar to that observed in Gram-negative bacteria, such as *Escherichia coli* [10–12]. No morphological evidence has been obtained for the presence of a component equivalent to a cell wall or peptidoglycan layer [8,9]. Recent studies using isolated endosymbionts from *C. deanei* showed the presence of two penicillin-binding proteins (PBP) in the envelope of these

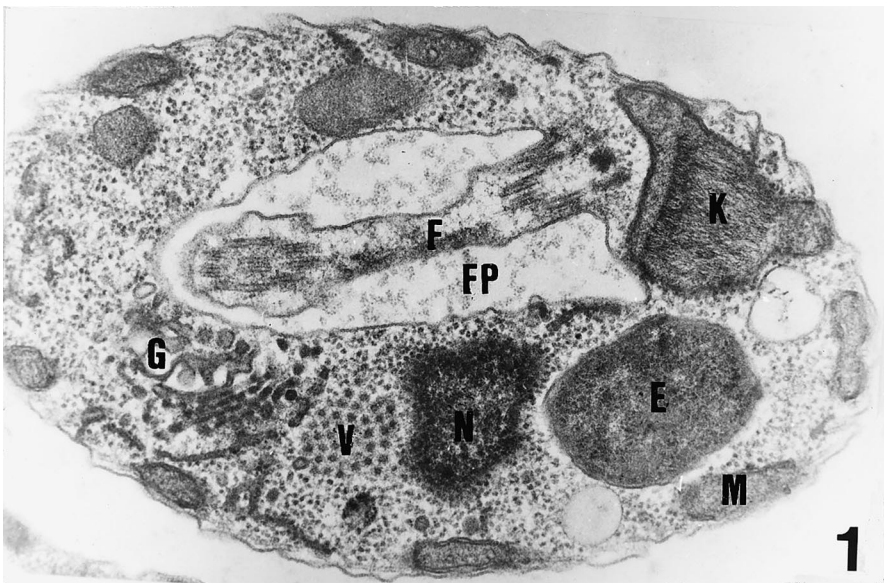

Fig. 1. Thin section showing the general aspect of *Crithidia desouzai*. The Kinetoplast (K), the nucleus (N), Golgi complex (G), flagellum (F), flagellar pocket (FP) and portions of the mitochondrion (M) located at the periphery of the protozoa can be seen. The endosymbiotic bacterium (E) and the aggregate of virus-like particles (V) in hexagonal array are evident. ×26 000.

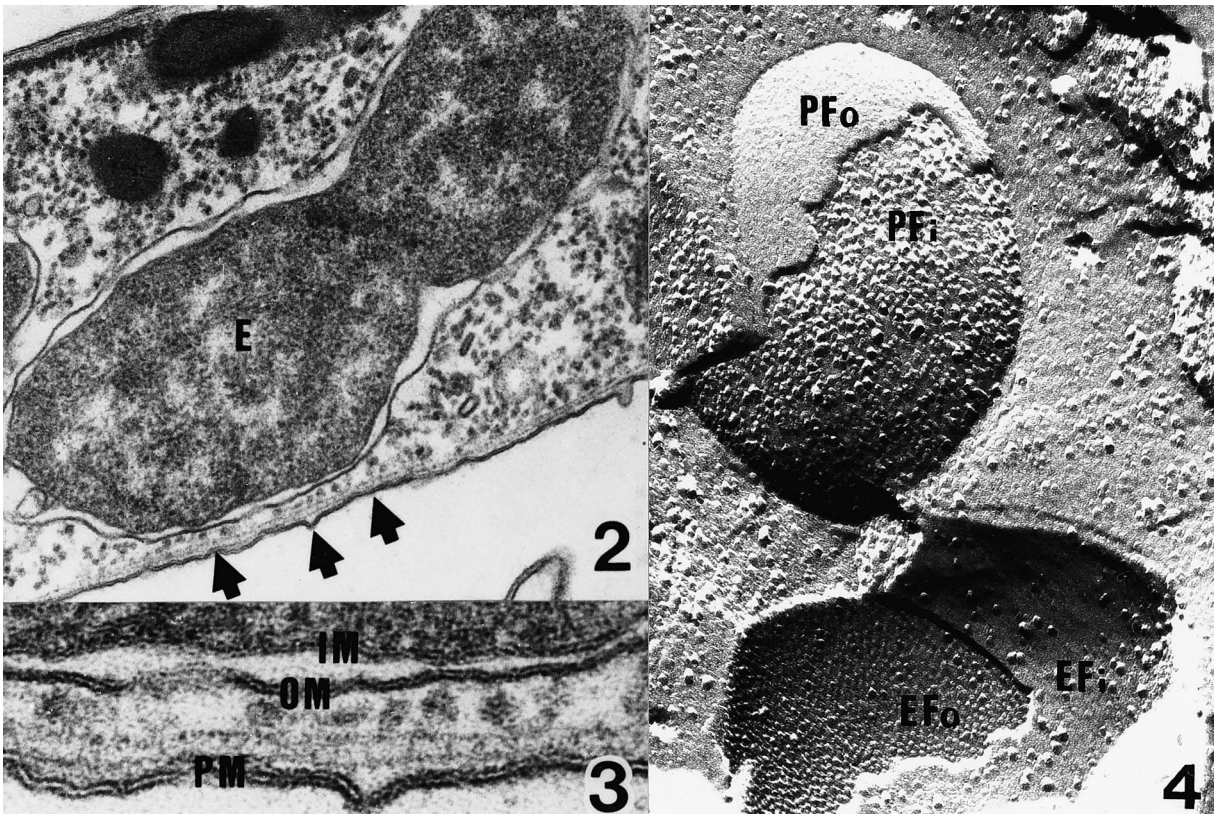

Fig. 2. Transmission electron microscopy of ultrathin sections. An initial stage of the division of the endosymbiont (E), which occurs by a constriction in the middle of the body. The endosymbiont is enveloped by two unit membranes, separated by an electron lucid space. ×40 000.

Fig. 3. Transmission electron microscopy of ultrathin sections. An enlarged view of the area, indicated by arrows in Fig. 2. The protozoan plasma membrane (PM) is thicker than the two membranes which surround the endosymbiont. IM, inner membrane of symbiont; OM, outer membrane of endosymbiont. ×15 000.

Fig. 4. Freeze-fracture image showing the four faces of the membrane of the symbiont of *C. desouzai*. PFi, protoplasmic face of the inner membrane; EFi, erternal face of the inner membrane; PFo, protoplasmic face of the outer membrane; EFo, external face of the outer membrane. ×64 000.

microorganisms with apparent molecular weights of 90 and 45 kDa [13]. The treatment with β-lactam antibiotics, leads to morphological alterations in the endosymbionts, indicating that a reduced or degenerated cell wall may exist and play important physiological roles in the division process and in morphological integrity maintenance [13]. The endosymbiont matrix has two distinct areas, one electron dense and the other electron lucid. The former is mainly composed of ribosomes of 15–18 nm diameter while the latter presents DNA filaments [6–8].

3. Isolation

An important step for the proper characterization of endosymbionts would be their isolation and cultivation. Cell fractioning of trypanosomatids is always a difficult task to perform. There are problems with their being disrupted under conditions favoring the preservation of intracellular structures, mainly due to the presence of a subpellicular microtubular layer. A few attempts have been made to obtain a purified fraction of endosymbionts; the trypanosomatids

were disrupted either by sonication or immunolysis, followed by differential centrifugation using sucrose or Percoll gradients [13–16]. Fractions are obtained which are considerably enriched in endosymbionts. Even though they seem to be generally well preserved, the maintenance of the endosymbionts in culture has never been obtained.

4. Obtainment of aposymbiotic strains

Successful obtainment of trypanosomatid aposymbiotic strains opened up new possibilities for experimental studies. Bruesk [5] was the first to use chloramphenicol for the purpose of curing *B. culicis*. The same approach has been used by other authors, so that at present, cultures of wild and aposymbiotic strains of *B. culicis*, *C. deanei*, *C. oncopelti*, and *Herpetomonas roitmani* are available. Recently Motta et al. [13] carried out a detailed study on the effect the of β-lactam antibiotics, ampicillin and cephalexin, on the *C. deanei* endosymbiont. Electron microscopic analysis showed that the endosymbionts suffered a progressive shape distortion process, leading to the appearance of pleomorphic forms.

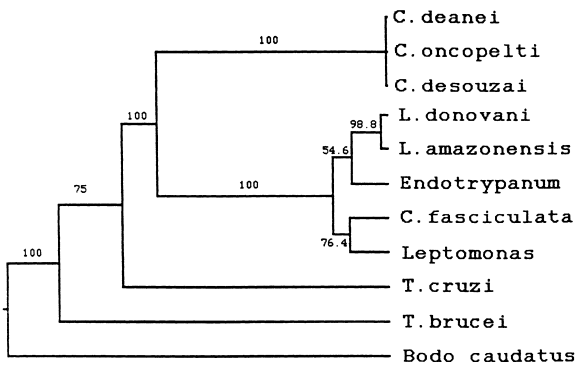

Fig. 5. Phylogenetic positions of *Crithidia oncopelti*, *C. deanei* and *C. desouzai* in the trypanosomatid family. The complete sequence or 730 informative sites were analyzed by DNApars, DNAcomp or DNApenny of PHYLIP, all giving the same topology. The tree presented was constructed using the distance matrix generated by UPGMA method in the Neighbor-Joining program of PHYLIP. The GenBank accession numbers beginning from the sequences of the three *Crithidia* spp. are: L29264, X07773, X53912,X53911, X03450, X53914, M31432, M12676, X53910.

5. Phylogenetic classification of the endosymbionts and their host cells

Electrophoretic isoenzymatic analyses were used to construct a trypanosomatid cladogram in which endosymbiont-containing *Crithidia* formed a separated group [17]. These data were further supported by SSU r-RNA analyses, where *Crithidia* species which harbor symbionts composed a independent phylogenetic branch, separated from *C. fasciculata* and other trypanosomatids (Fig. 5).

With regard to the phylogenetic classification, endosymbionts present in *Crithidia* species and in *B. culicis* showed a great similarity and are close to *Bordetella bronchiseptica*. The symbionts found in *Crithidia* and *Blastocrithidia* were thus designated, respectively, as *Kinetoplastibacterium crithidii* and *Kinetoplastibacterium blastocrithidii*. These microorganisms were grouped together with bacteria of the genera *Bordetella*, *Neisseria* and *Eikenella*, in the β division of Proteobacteria [18,19].

6. Endosymbiont–protozoan relationship

The available data indicate that the presence of the endosymbiont induces morphological and biochemical changes in the host trypanosomatid. The endosymbiont divides synchronously with the host protozoa; thus at the end of the mitotic process, each daughter cell contains a single symbiotic microorganism.

A recent three-dimensional reconstruction of *C. deanei* [20] clearly showed that glycosomes, a special type of peroxisome where most of the glycolytic enzymes of the trypanosomatids are located, are closely associated with the endosymbiont (Figs. 6 and 7).

A characteristic feature of the trypanosomatids is the presence of a paraxial or paraflagellar structure located in the flagellum. It is formed by a complex array of filaments of several types made of at least two major proteins with 7 and 25 nm of thickness [21]. It has been observed that the paraxial rod is not found in trypanosomatids that harbor endosymbiont [22], suggesting that the presence of the microorganism inhibits the expression of the genes coding for proteins of that structure. The presence of the endosymbiont also interferes with the spatial distribution

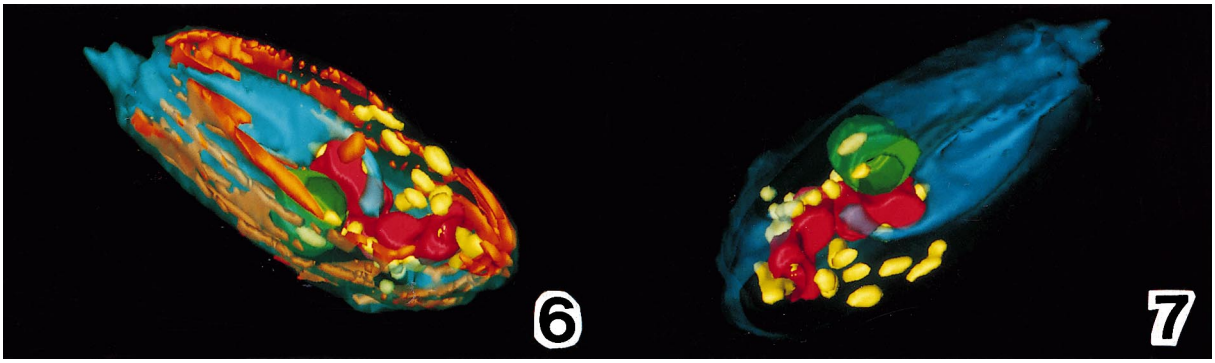

Fig. 6. Three dimensional reconstruction of 12 planes of *C. deanei*. Membrane (blue), nucleus (green), mitochondrion (orange), glycosomes (yellow) and endosymbiont (magenta). Note that the mitochondrion is seen as a unique, branched organelle, which underlies the plasma membrane.

Fig. 7. Three dimensional reconstruction of 12 planes of *C. deanei*. Membrane (blue), nucleus (green), mitochondrion (orange), glycosomes (yellow) and endosymbiont (magenta). The mitochondrion was omitted from the final image for a clearer observation of the association between the endosymbiont (magenta) and the glycosomes (yellow).

of the microtubules and the mitochondrion. At some points of the cell surface, the subpellicular microtubules are displaced from their typical sub-pellicular localization and portions of the branched mitochondria touch the inner portion of the plasma membrane. A third significant change occurs with the kinetoplast DNA network. In trypanosomatids of the genera *Crithidia*, *Blastocrithidia* and *Herpetomonas*, the kinetoplast DNA (k-DNA) is usually tightly packed, forming a compact disk-like structure. In endosymbiont-bearing trypanosomatids the k-DNA fibers are more dispersed, forming a looser network. Removal of the endosymbiont by antibiotic treatment did not reverse all these effects [22].

The presence of the endosymbiont interferes with the metabolism of the trypanosomatid. Lwoff [23] reported that *C. oncopelti* could be easily grown in a medium containing peptone and supplemented with thiamine. In addition, contrary to what was known for the other trypanosomatids, *C. oncopelti* could be cultivated in a medium without hemin. Later on, Newton [24] analyzed, in more detail, the nutritional requirements of *C. oncopelti* and established a chemically defined medium containing a high essential amino acid, methionine, vitamins, such as thiamine and nicotinamine, *p*-aminobenzoic acid, glucose, salts and some trace elements. After the production of aposymbiotic strains, it became clear that only endosymbiont-containing cells could be cultivated in a medium with no hemin. In the case of *C. deanei*, Mundim et al. [25] reported that it could be grown in a chemically defined medium containing two amino acids, methionine and tyrosine, four vitamins (folic acid, thiamine, biotin and nicotinamide), and no source of hemin and purine. Indeed, the ability to grow in a chemically defined medium is now being routinely used for the detection of new trypanosomatid isolates containing endosymbiont. It has been shown that enzymes and precursors which participate in the heme compounds metabolic pathway are more abundant in normal than in aposymbiotic strains [26]. It is well known that the trypanosomatids are not able to synthesize the tetrapyrrol group, and so need hemin, hematin or hemoglobin in the culture medium. The presence of uroporphyrinogen synthase I in the endosymbiont would, in part, explain the fact that endosymbiont-containing strains do not require these substances in the medium [27]. The endosymbiont is also involved in ornithine metabolism since trypanosomatids containing this microorganism do not need ornithine, arginine and citrulline in the culture medium. The endosymbiont contains acetylornithinase, an enzyme involved in the formation of ornithine from acetylor-

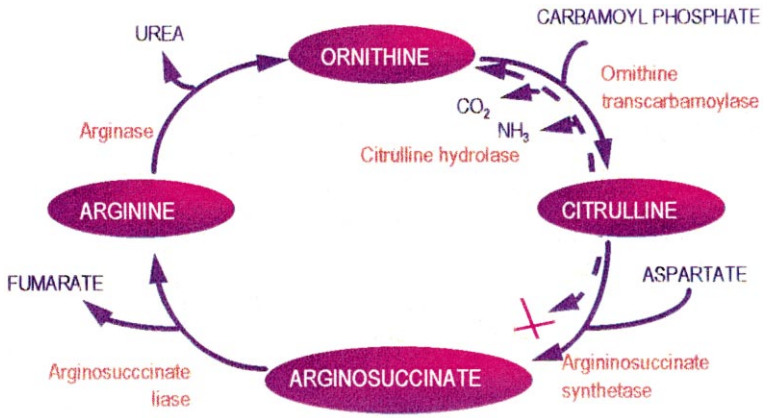

Fig. 8. Urea cycle in endosymbiont-containing and non-containing *Crithidia* species. Full arrows indicate the metabolic pathways which are present in both strains. The dashed arrows represent the urea cycle as occurs in symbiont-free strains. Note the presence of ornithine transcarbamoylase closing the cycle in endosymbiont-bearing strains.

nithine. Ornithine transcarbamoylase, which transforms ornithine in citrulline (Fig. 8), could be cytochemically localized in the endosymbiont [28,29]. Endosymbiont-bearing trypanosomatids also grow in a medium without isoleucine, valine and leucine due to the presence in the symbiont of threonine deaminase, the first enzyme in the biosynthetic pathway to isoleucine from threonine [14].

Taken together, these observations suggest that several metabolites important for the trypanosomatid growth are synthesized by the endosymbiont. Thereby, complete metabolic cycles are established between the prokaryont and the host cell. By definition, in a true symbiotic relationship both elements take advantage of the association.

Recent studies using conventional transmission electron microscopy and 3-D reconstruction in *C. deanei* suggest a close association between the endosymbiont and glycosomes. Ultrastructural cytochemical observations indicate that the type aa_3 cytochrome oxidase is inactive or absent in the endosymbiont respiratory chain. Cytochemical assays to detect the succinate dehydrogenase activity also suggest that the endosymbiont in *C. deanei* probably has an inactive respiratory chain. These data were reinforced by the low enzymatic activity of succinate cytochrome *c* reductase found in an enriched symbiont fraction. It is, therefore, possible that part of the ATP produced in the mitochondrion and in the glycosomes of the protozoan are used by the endosymbiont. An ATPase activity, probably localized on the surface of the endosymbiont, has been detected. Perhaps this enzyme is used for hydrolysis of ATP produced by the host protozoan. In this way, an endosymbiont energized membrane is generated where a proton motive force could maintain a transport mechanism permitting the endosymbiont to obtain compounds, such as ions, adenosine, etc., from the protozoan cytoplasm [20].

The presence of the endosymbiont also interferes with surface properties of the protozoan, such as: (a) exposition of carbohydrate residues, as evaluated by interaction with lectins [30–32] and by identification of membrane-associated polysaccharides using gas–liquid chromatography [33]; and (b) the surface charge, as evaluated by the binding of cationic particles to the protozoan surface and direct measurement of its electrophoretic mobility [34].

The presence of the endosymbiont seems to interfere also with the life cycle of the trypanosomatid. At least in the case of *H. roitmani*, endosymbiont elimination by chloramphenicol treatment significantly increased the process of differentiation of promastigote into opistomastigote forms [33].

There is clear evidence indicating that some organelles found in eukaryotic cells, such as mitochondria and chloroplasts, originated from microorganisms which established a symbiotic relationship with the host cell [35–38]. Some of the symbiont genes were possibly transferred to the host during the evolutionary process. It seems thus that the endosymbiont–trypanosomatid system, which can easily be main-

tained in a chemically defined medium, may be a useful model to study basic processes of the endosymbiont–host cell relationship and the origin of new organelles.

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