The Reservosome of *Trypanosoma cruzi* Epimastigotes: an Organelle of the Endocytic Pathway with a Role on Metacyclogenesis

Maurilio José Soares

Laboratório de Biologia Celular de Microrganismos, Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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Reservosomes are large (0.4-0.6 μm in diameter) membrane-bound organelles found at the posterior end of *Trypanosoma cruzi* epimastigote forms. They present a protein-rich, electron dense matrix, where several tiny round electron lucent inclusions are immersed. Due to their inner structure, these organelles were formerly designated as multivesicular bodies (De Souza 1984). However, ultrastructural demonstration that the inner inclusions were not membrane bound, together with cytochemical evidentiation that these vesicles were indeed lipid droplets, revealed the misinterpretation of the former nomenclature, and the name reservosome was then proposed (Soares & De Souza 1988). Reservosomes are morphologically and biochemically distinct from the recently described acidocalcisomes (Scott et al. 1997). As reservosomes are a site for protein accumulation, two major questions arise: how proteins arrive there, and what reservosomes are for?

THE ENDOCYTIC PATHWAY

Endocytosis of nutrients in trypanosomatid protozoa is restricted to the flagellar pocket membrane (Webster & Russel 1993, Radek & Hausmann 1994, Overath et al. 1997). However, epimastigotes of *T. cruzi* present an additional site for the uptake of macromolecules: the cytostome, a deep invagination of the cell plasma membrane close to the flagellar pocket region. It appears that the cytostome is physically linked to the flagellum (Okuda et al. 1977) and represents the main site for both receptor-mediated-endocytosis and fluid-phase-pinocytosis in epimastigotes (Soares & De Souza 1991, Porto-Carreiro et al. 1998). Endocytic vesicles bud off from the cytostome and the flagellar pocket membranes and then deliver their cargo to the reservosomes (Soares & De Souza 1991, Soares et al. 1992). Reservosomes contain tyrosine-phosphorylated proteins, suggesting that protein kinases play a role in the internalization process (Vieira et al. 1996). Incubation of epimastigotes with ATP (50 mM, for 24 hr) prior to the addition of horseradish peroxidase (as a marker of the endocytic pathway) affected the formation of normal reservosomes (Bogitsh et al. 1997), possibly acting on the (still unknown) translocation system governing the traffic of the small endocytic vesicles that cargo proteins from the cell surface to the storage organelles.

Reservosomes are acidic organelles, containing cruzipain (a cysteine proteinase) and ingested proteins. On this basis, it has been proposed that these structures are pre-lysosomal compartments (Soares et al. 1992). Imunocytochemical quantification using DAMP as a probe showed that reservosomes have a luminal pH of about 6.0 (Soares et al. 1992). However, it is still a matter of speculation how these organelles are acidified, as they were not labeled with antibodies against a vacuolar-type H^+-ATPase, which however recognized other intracellular vacuoles, possibly the acidocalcisomes (Benchimol et al. 1998). A 52-kDa protein sharing sequence homology with glutathione S-transferase (Tc52) has been also localized in reservosomes (Ouassi et al. 1995). It has been postulated that Tc52 is released from the parasite to the external milieu, in order to scavenge glutathione (GSH). The Tc52-GSH complex could be then internalized (by receptor mediated endocytosis?) and accumulated in the reservosomes. As GSH may serve as a storage and transport form of cysteine moieties, it was suggested that the Tc52-GSH complexes might act as a cysteine delivery system. Accordingly, TC52 is developmentally regulated, being fully expressed only by the epimastigotes.

The presence of an early endosomal compartment in *T. cruzi* epimastigotes is still controversial. It is well known that incubation of the para-

Fax: +55-21-260.4434. E-mail: maurilio@ioc.fiocruz.br
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sites at 28°C with gold labeled proteins results in labeling inside cytoplasmic vesicles and tubules, as well as in the reservosomes (Soares & De Souza 1991, Soares et al. 1992). Figueiredo and Soares (1996) showed that incubation of the parasites at 12°C (a condition that hinders the fusion of endocytic vesicles with early endosomes in mammalian cells) blocked the pinching of endocytic vesicles at the cytostome, inhibiting the uptake of nutrients by the cells. Labeling could be found in the cytostome, but not inside the flagellar pocket or intracytoplasmic vesicles. When the temperature was raised to 28°C, labeling could be then again found in the reservosomes. From these experiments, it was concluded that early endosomes are lacking; cargo vesicles coming from the cell surface (cytostome and flagellar pocket membranes) should shuttle their content directly to the reservosomes. On the other hand, three-dimensional reconstruction of cytoplasmic tubules and vesicles located close to the flagellar pocket showed that they are interconnected, forming a branched network at the anterior end of the cell, morphologically similar to the typical mammalian early endosomes (Porto-Carreiro et al. 1998).

Bogitsh et al. (1996) presented some data demonstrating that, although containing cysteine proteinase, reservosomes are unlikely to be lysosomes (albeit lysosomes have not yet been clearly morphologically and biochemically defined in trypanosomatids). The authors showed that incubation of epimastigotes with ammonium chloride (a weak base that accumulates in acidic compartments) resulted in swelling of reservosomes and electron-lucent vacuoles (considered as lysosomes). However, the exposure period required for swelling of reservosomes was significantly greater than that required for the same effect in lysosomes, probably due to the different pH inside these compartments. Furthermore, methyl esters of aminoacids (which accumulate in eukaryotic lysosomes) had little effect upon reservosomes, precluding their being lysosomes and suggesting that the proteolytic enzymes, such as cysteine proteinases, can be in an inactive state during a life period of the parasites.

THE ROLE OF RESERVOSOMES IN METACYCLOGENESIS

A stereological study showed that reservosomes occupy about 6% of the total cell volume of epimastigotes, but gradually vanish during the differentiation process to the trypomastigote form (Soares et al. 1989, Figueiredo et al. 1994). It has been suggested that the nutrients accumulated in the reservosomes could be used as a main energy source for this activity. A fascinating hypothesis is that a nutritional stress triggers the acidification of the luminal content and the activation of the enzymes contained inside the reservosomes, which then evolve to a lysosomal state. Degradation of stored proteins would then lead to the disappearance of the reservosomes, with the release of amino acids to the cell cytoplasm. Accordingly, biochemical data demonstrate that consumption of amino acids is favored in epimastigotes under starvation conditions (Urbina 1994).

Reservosomes contain cruzipain (also known as cruzain and GP57/51), the major cysteine proteinase of *T. cruzi*. Expression of cruzipain is developmentally regulated, the enzyme levels being about 10-fold higher in epimastigotes (Cazzulo et al. 1997). High expression of reservosomes and cysteine proteinases in epimastigotes, but not in trypomastigotes, suggests the participation of reservosomes in *T. cruzi* metacyclogenesis. Some data support this hypothesis: Franke de Cazzulo et al. (1994) demonstrated that proteinase inhibitors reduced growth and differentiation of *T. cruzi*. Ultrastructural data showed that treatment of the parasites with cysteine proteinase inhibitors arrested the transport of the enzymes to the reservosomes at the Golgi complex cisternae level, leading to cell death (Engel et al. 1998). Preliminary observations reported by Figueiredo et al. (1998) in epimastigotes maintained in TAU3AAG medium (a condition that induces metacyclogenesis) showed that a close relationship exists between uptake of nutrients, adhesion to the substrate and cell differentiation in *T. cruzi*.

CONCLUSION

Although the reservosome seems to play a pivotal role in the life cycle of *T. cruzi*, little is still known about this fascinating organelle. A still blurry image is slowly coming to sight relative to the endocytic process of *T. cruzi* cells, but unfortunately most data has been obtained from epimastigote forms maintained in culture media. A more precise characterization of reservosomes and cytoplasmic vesicles is at the moment difficult, since specific markers to the endocytic compartments are still lacking. Some hope comes from the recent obtention of a purified subcellular fraction containing reservosomes of T. *cruzi* epimastigotes (Cunha-e-Silva et al. 1998). Involvement of this organelle in vital metabolic pathways of the parasites indicates that reservosomes are potential targets for the development of chemotherapeutic drugs.

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