ELSEVIER

Institut Pasteur

Microbes and Infection xx (2017) 1–6

www.elsevier.com/locate/micinf

Short communication

# A structural analysis of the natural egress of *Toxoplasma gondii*

Lúcio Ayres Caldas [a,*], Marcia Attias [a], Wanderley de Souza [a,b]

[a] *Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil*
[b] *Instituto Nacional de Ciência e Tecnologia de Biologia Estrutural e Bioimagem, Rio de Janeiro, Brazil*

Received 22 June 2017; accepted 11 September 2017
Available online ■ ■ ■

**Abstract**

Previous studies have analysed the process of *Toxoplasma gondii* egress with the aid of inducers, such as calcium ionophores. Although calcium transients have been successful in triggering *T. gondii* egress, the structural panorama of "natural" and artificial events should match. The present study approaches the natural egress of this parasite using super-resolution and electron microscopy and reveals lytic and non-lytic events of individual egress; this corroborates the use of calcium ionophore as a reliable tool to trigger parasite egress. Altogether, our data suggest that different signalling routes can converge to similar structural aspects in natural and induced egress.


*Keywords:* Parasite interactions; Egress; Toxoplasma microscopy

## 1. Introduction

*Toxoplasma gondii* is an intracellular protozoan that is able to infect a large number of cell types. Significant efforts have been invested on the understanding of basic aspects of its cycle, especially the steps of motility, invasion and division. In contrast, there is still little information on the process of evasion from the host cells. It is well known that parasite internalization is preceded by parasite adhesion, reorientation and then secretion of its apical organelles. Micronemes, rhoptries and dense granule discharge is crucial for invasion and the formation and development of a non-fusogenic parasitophorous vacuole (PV), which is a chimerical wrapping to the new environment in which replication occurs [1–4]. This PV is moved to the perinuclear host cell locus, usually in association with host cell mitochondria and endoplasmic reticulum [5], and parasites replicate relatively protected from the host immunological system (reviewed in Ref. [6]).

Previous studies performed by Moudy et al. [7] indicated that, after several rounds of division, the mechanical stress caused by the PV's growth resulted in micro-ruptures in the host cell plasma membrane, leading to an ionic imbalance that would trigger *T. gondii* mobility and egress from the host cell in a way that was suggested to resemble the invasion process in some aspects [8]. Subsequent studies have shown that the PV rupture was not mechanically triggered and that the parasites secrete perforins that act on the permeabilization and fragilization of the PV [9]. Besides that, tachyzoites were released from individual vacuoles under artificial stimuli [10]. This late stage of the *T. gondii* cell cycle, however, is still poorly understood, and the knowledge in this field was largely provided by the seminal studies of Endo et al. [11] that successfully synchronized the egress of tachyzoites using calcium ionophore.

A few studies on *T. gondii* egress were performed that induced it with ethanol [12], potassium ionophore [13] and recently the phosphodiesterase inhibitor zaprinast [14] among others; however, the calcium ionophore A23187 has been used by most groups [11,15–19]. Although effective, it still is an artificial method that provokes a rise in the concentration of cytoplasmic calcium in the host cell. Investigation of natural

* Corresponding author. Laboratório de Ultraestrutura Celular Hertha Meyer, Av. Carlos Chagas Filho 373, Prédio CCS, Bloco C, Subsolo, Cidade Universitária, Rio de Janeiro, CEP:21941902, RJ, Brazil.
*E-mail address:* lucio@biof.ufrj.br (L.A. Caldas).

https://doi.org/10.1016/j.micinf.2017.09.006

egress *in vitro* can provide clues on the fidelity and reliability of the use of calcium ionophore as a method to efficiently trigger *T. gondii* egress, mimicking "natural" conditions.

In this short communication, we employ super-resolution fluorescence microscopy, transmission and scanning electron microscopy to describe the natural egress of *T. gondii* from host cells. The results show that with and without the use of inducers, the parasite egress process is structurally similar.

## 2. Materials and methods

### 2.1. Parasites and host cell culture

The RH wild-type *T. gondii* tachyzoites used were obtained by a peritoneal washing of 2–3 day-infected mice, then centrifuged at 1000 g for 10 min to remove cell debris and peritoneal leukocytes following quantification of parasites in the supernatant using a Neubauer chamber. The parasites were resuspended in Dulbecco's modified Eagle's medium (DMEM). Swiss mice were bred at the Universidade Estadual do Norte Fluminense animal facility. The experimental protocol was approved by the Instituto de Biofisica Carlos Chagas Filho (Universidade Federal do Rio de Janeiro) Ethics Committee for animal experimentation.

The host cells were *Macaca mulatta* kidney cells (LLC-MK2) maintained *in vitro* in DMEM supplemented with 10% foetal bovine serum (FBS) at 37 °C in a 5% $CO_2$ atmosphere.

### 2.2. In vitro *infection*

Incubation of parasites was performed at a ratio of 10 parasites per cell during 40–50 min at 37 °C and 5% $CO_2$. Then, supernatant containing the free parasites was aspirated and replaced by fresh medium supplemented with 5% FBS, and the infection was allowed to proceed for determined times.

### 2.3. Video microscopy

Confluent monolayers of LLC-MK2 cells that were previously adhered to a glass coverslip were infected with *T. gondii*; they were then incubated and monitored using a long-term time-lapse with a Nikon BioStation IM.

### 2.4. Super-resolution fluorescence microscopy

For immunofluorescence microscopy, the cells were seeded onto round coverslips, infected and, at determinate hours post-infection (hpi), processed as described by Ref. [20]. Primary monoclonal antibody (mouse anti-tubulin-α B-5-1-2 from Sigma–Aldrich) and secondary (goat anti-mouse IgG conjugated to Alexa Fluor 488) antibodies were incubated at 1:100 and 1:400 dilution, respectively, for 1 h. Phalloidin (red) (Sigma–Aldrich) was used to label actin filaments. It was diluted 1:40 and incubated for 20 min at room temperature. Images were performed in a Zeiss Elyra PS.1, using Super Resolution Structured Illumination Microscopy (SR-SIM) mode. We employed 5 phases and 5 rotations, and generated image's algorithms were solved by ZEISS software ZEN 2012 (version 9.1.1.5).

### 2.5. Electron microscopy

For field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM), samples were processed as described in Ref. [17]. A subset of the samples was dry-cleaved with carbon adhesive tape prior to sputtering to expose the interior of the cell, as described in Ref. [21].

For immunolabeling, the samples were permeabilized with 0.1% Triton-X-100 for 2 min prior to fixation with 0.1% glutaraldehyde and 4% freshly prepared formaldehyde. The samples were then probed with rabbit anti-actin antibody at a 1:100 dilution, followed by a goat-anti-rabbit antibody conjugated to 10 nm colloidal gold particles at a 1:400 dilution. Both the antibodies were incubated for 1 h, processed for FESEM, and observed using an electron-backscatter detector. The samples that were to undergo scanning electron microscopy were observed in a JEOL 6340 FESEM (5 kV; WD 8 mm) or a ZEISS Auriga 40 (1 kV; WD 3 mm). The following transmission electron microscopes were used: Zeiss 900 and Tecnai Spirit 120 – FEI.

## 3. Results

Interaction times between 24 and 48 hpi were chosen in an attempt to document *T. gondii* egress – from its pinching off from the rosette arrangement to its arrival at the extracellular ambient. The general process was documented by video microscopy of the ~48 hpi naturally occurring egress (Supplemental Material 1) where cell lysis seems to occur along with the first movements of PV-resident tachyzoites.

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.micinf.2017.09.006.

One of the first structural signals that the egress process has started is when intracellular parasites start to move. Fig. 1A and B correspond, respectively, to the transmission electron microscopy and a Z-stack image in the SIM mode of super-resolution fluorescence microscopy of the host cells at 48 hpi. In both images, tachyzoites were still organized in their rosette arrangement within the PVs. The host cell and *T. gondii* subpellicular microtubules were labelled in Fig. 1B and C; the latter represents the next step of egress when a group of parasites detaches from the rosette and starts to exit from the PV. It is noteworthy that while the presence of host cell microtubules around the PV became very evident, the host cell actin stretched disposition (Fig. 1B) changes during the *T. gondii* egress (Fig. 1C), which can be a signal of host cell detachment from the substrate. The overlaid image from the adhesive tape scraping 48 hpi cells by FESEM (Fig. 1D) shows that the detachment of parasites from the rosette is not synchronic.

After escaping the PV, parasites glide through the host cell cytosol, where they should face diverse intracellular contents, such as the host cell cytoskeleton elements. Backscattering electrons from FESEM showed host cell actin (Fig. 1E) and



Please cite this article in press as: Caldas LA, et al., A structural analysis of the natural egress of *Toxoplasma gondii*, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.09.006