Cryptococcosis caused by the encapsulated yeast *Cryptococcus neoformans* affects mostly immunocompromised individuals and is a frequent neurological complication in AIDS patients. Recent studies support the idea that intracellular survival of *Cryptococcus* yeast cells is important for its pathogenesis. However, the initial steps of *Cryptococcus* internalization by host cells remain poorly understood.

We investigated the mechanism of *C. neoformans* phagocytosis by peritoneal macrophages using laser scanning confocal microscopy (LSCM) and electron microscopy techniques, as well as flow cytometry (FACS) quantification. Five *C. neoformans* strains differing in serotypes and capsule size - H99, B3501, ATCC28957 and acapsular mutants CAP59 and CAP67 - were allowed to interact with peritoneal macrophages, previously adhered on glass cover slips, in a ratio of 25:1 yeast per macrophage, for 2 hours. Interactions occurred in the presence or absence (control) of cytoskeletal dynamics inhibitors: cytochalasin D, latrunculin B, nocodazole or placlitaxel and then cells were processed for FACS, LSCM and transmission and scanning electron microscopy (TEM and SEM, respectively). For LSCM, actin filaments were labeled with AlexaFluor® 488 phalloidin and α-tubulin was labeled with anti-α-tubulin AlexaFluor® 546 conjugate.

Electron microscopy analyses revealed that capsular and acapsular strains of *C. neoformans* are internalized by macrophages via both ‘zipper’ (receptor-mediated) and ‘trigger’ (membrane ruffle-dependent) phagocytosis mechanisms (Fig.1). Actin filaments surrounded phagosomes of capsular and acapsular yeasts (Fig. 2), and the actin depolymerizing drugs inhibited yeast internalization and actin recruitment to the phagosome area. In contrast, inhibitors of microtubule dynamics decreased internalization but did not prevent actin recruitment to the site of phagocytosis.

Our results show that different uptake mechanisms, dependent on both actin and tubulin dynamics occur during yeast internalization by macrophages, and that capsule production does not affect the mode of *Cryptococcus* uptake by host cells.

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Fig. 1: Uptake of Cryptococcus neoformans acapsular strain CAP59 after interaction with peritoneal macrophages. Scanning electron microscopy showed both trigger-like (A) and zipper-like (B) uptake structures. Scale bars represent 1 μm (A) and 2 μm (B).

Fig. 2: Actin is recruited to the phagosome area during yeast uptake. Confocal laser scanning microscopy of internalized Cryptococcus neoformans strain H99 identified by bright field (arrow in A) can be visualized in the context of host cell actin (red) and microtubule (green) cytoskeletons. Host cell DNA is labeled with DAPI (blue). Bar represents 5μm.