Protein kinase D and cortactin are recruited to actin-rich cup-like structures formed during *Trypanosoma cruzi* extracellular amastigotes entry into HeLa cells: unveiling novel host cell-signaling pathways modulated by the parasites

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Extracellular amastigote forms (EA) of *Trypanosoma cruzi* depends on actin host cell polymerization to invade. Protein Kinase D (PKD) is a family of multidomain enzymes (PKD1, 2 and 3). PKD lies downstream of PKCs in a novel signal transduction pathway implicated in the regulation of multiple fundamental biological processes. At the leading edge of migrating cells active PKD co-localizes with F-actin, Arp2/3 and cortactin. Cortactin has emerged as a key signaling protein in cellular processes such as endocytosis and tumor invasion, by interacting/altering cortical actin network. PKD is an upstream regulator of cortactin. We aimed to evaluate the role of PKD and cortactin in EA uptake by HeLa cells. The recruitment of cortactin and PKD-GFP-vectors by EA in HeLa cells was assessed both in vitro (fixed cells and indirect immunofluorescence) and in vivo (live-cell imaging by time-lapsed confocal microscopy). Wild type PKD1 and 2, but not PKD3 are recruited with cortactin to sites of actin-rich cup-like structures induced by EA invasion. Parasites killed by heat or epimastigotes (non-infective forms) recruit none of the proteins. Kinetic assays showed that actin is recruited earlier than cortactin and PKD. PKD1 lacking N-terminal domain is recruited to and colocalizes with actin and cortactin in EA invasion sites, whilst PKD1 pleckstrin homology-deleted and PKD1-kinase-dead are not. These results strong suggest that unexpected novel roads may also be explored by *Trypanosoma cruzi* to invade cells. We are currently knocking down cortactin and PKD proteins in HeLa cells in order to examine their requirement for EA *T. cruzi* invasion.

Keywords: *Trypanosoma cruzi*, Protein kinase D, cortactin, invasion