**Entamoeba histolytica** and pathogenesis: A calcium connection

Mrigya Babuta¹, Sudha Bhattacharya², Alok Bhattacharya³*

¹ School of Life Sciences, Jawaharlal Nehru University, New Delhi, India, ² School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India, ³ Department of Biology, Ashoka University, Sonepat, Haryana, India

Current address: Beth Israel Deaconess Medical Centre, Department of Medicine, Division of Gastroenterology, Centre for Life Science, Boston, Massachusetts, United States of America

* alok.bhattacharya@ashoka.edu.in

**Abstract**

Calcium signaling plays a key role in many essential processes in almost all eukaryotic systems. It is believed that it may also be an important signaling system of the protist parasite **Entamoeba histolytica**. Motility, adhesion, cytolyis, and phagocytosis/trocytosis are important steps in invasion and pathogenesis of **E. histolytica**, and Ca²⁺ signaling is thought to be associated with these processes leading to tissue invasion. There are a large number of Ca²⁺-binding proteins (CaBPs) in **E. histolytica**, and a number of these proteins appear to be associated with different steps in pathogenesis. The genome encodes 27 EF-hand-containing CaBPs in addition to a number of other Ca²⁺-binding domain/motif-containing proteins, which suggest intricate calcium signaling network in this parasite. Unlike other eukaryotes, a typical calmodulin-like protein has not been seen in **E. histolytica**. Though none of the CaBPs display sequence similarity with a typical calmodulin, extensive structural similarity has been seen in spite of lack of significant functional overlap with that of typical calmodulins. One of the unique features observed in **E. histolytica** is the identification of CaBPs (EhCaBP1, EhCaBP3) that have the ability to directly bind actin and modulate actin dynamics. Direct interaction of CaBPs with actin has not been seen in any other system. Pseudopod formation and phagocytosis are some of the processes that require actin dynamics, and some of the amoebic CaBPs (EhC2P, EhCaBP1, EhCaBP3, EhCaBP5) participate in this process. None of these **E. histolytica** CaBPs have any homolog in organisms other than different species of Entamoeba, suggesting a novel Ca²⁺ signaling pathway that has evolved in this genus.

**Introduction**

The protist parasite **Entamoeba histolytica** causes human amebiasis, a major public health problem in developing countries. Though great strides have been made in understanding the pathobiological mechanisms of the disease in the last few decades, details about the molecular pathways that are involved in tissue invasion and damage during both intestinal and extraintestinal diseases are not clear. Because only a fraction of infected individuals (about 10%)
display invasive disease, an understanding of the signaling system that triggers invasion by the parasite is needed for the development of better therapeutic molecules. Clear linkage between the genotype of the parasite with invasive disease or with extraintestinal invasion has not been seen, though a number of virulence factors have been identified in recent years [1]. The host–parasite relationship in amebiasis is also modulated by host factors, which include host genes (such as leptin) and gut microflora [2]. Gut bacteria provide not only feeding material but also an anaerobic environment and pH conducive for the trophozoites to multiply and differentiate into cysts [3]. It is increasingly believed that the gut environment and parasite genotype, along with the host genotype, all interact to create the right environment for *E. histolytica* to invade [3,4]. However, we do not have any clear idea about the nature of these interactions and how these eventually influence the parasite’s ability to invade tissues.

**Ca\(^{2+}\) homeostatic mechanism in *E. histolytica***

Ca\(^{2+}\) is one of the versatile, ubiquitous second messengers that mediate pathways by altering the shape, charge, and electrostatic interaction of downstream effector molecules [5]. In order to mediate response in the presence of a stimulus, cells have developed a “signaling toolkit” to sequester or compartmentalize Ca\(^{2+}\) and release it as and when needed [6]. This toolkit comprises Ca\(^{2+}\)-mobilizing signals that regulate the level of Ca\(^{2+}\) in different cellular compartments by activating various ion channels and transporting systems. Once Ca\(^{2+}\) is released, a repertoire of CaBPs, Ca\(^{2+}\) buffers, and Ca\(^{2+}\)-regulated enzymes subtly translate these Ca\(^{2+}\) signals into a cellular response. After initiation of a response by activating the appropriate pathway, Ca\(^{2+}\) is rapidly removed from the cytoplasm by various pumps and exchangers [6]. It is not clear whether *E. histolytica* encodes most of the molecules needed for release and sequestration of Ca\(^{2+}\) in response to a signal. Only a handful of molecules have been reported. A figure summarizing our current understanding and the molecules involved is shown in Fig 1. There are 5 genes encoding putative Ca\(^{2+}\)-ATPases, out of which 3 belong to plasma membrane Ca\(^{2+}\)-ATPase (PMCA) and 2 to sarcoendoplasmic reticulum ATPase (SERCA), and these are present in vacuoles and in the cytoplasmic network, respectively [7,8]. More recently, 2 Ca\(^{2+}\)-ATPases from *E. histolytica* (Eh), namely EhSPCA (secretory pathway calcium ATPase) and EhCCX (Ca\(^{2+}\)/cation exchanger), have been identified. These are present on the membrane of some cytoplasmic vesicles [9,10]. Interestingly, overexpression of EhCCX enhanced the virulence and reduced the cell death of trophozoites [9].

*E. histolytica* has ionophore-releasable Ca\(^{2+}\), comprising around 70% of the total Ca\(^{2+}\) pool that can be divided into 2 parts. One is stimulated by the second messenger inositol 1,4,5-triphosphate (Ins(1,4,5)P3) releasing internal Ca\(^{2+}\) from endoplasmic reticulum-like structures [11]. The second one is sensitive to Ins(1,3,4,5)P4 [12]. Though it appears that both these second messengers act on 2 different Ca\(^{2+}\) stores, it is not clear whether there is a link between them in this organism. *E. histolytica* also encodes a calpain-like protein and many nucleotidases that require Ca\(^{2+}\), such as Ca\(^{2+}\)-dependent ATPase/ADPase, Ca\(^{2+}\)-dependent thiamine pyrophosphatase, and acid phosphatase. The calpain-like protein is thought to be associated with apoptosis of the parasite because its level is increased during programmed cell death of trophozoites. It was also found in the cytoplasm and near the nucleus [13,14], whereas some of the nucleotidase enzymes are present in the inner membrane of cytoplasmic vacuoles that may or may not be phagolysosomes [15–17]. It is also not clear whether these enzymes participate in calcium homeostasis in this organism. Genomic analysis identified a repertoire of 27 multi-EF-hand–containing CaBPs in *E. histolytica* [18]. Some of these proteins are suspected to be Ca\(^{2+}\) buffers, thereby participating in the regulation of Ca\(^{2+}\) concentration in different cellular compartments.
The role of Ca\(^{2+}\) in the pathogenesis of *E. histolytica*

The initial step during the process of invasion is adherence to the target cells after the contact has been made. A number of molecules that are involved in this process have been identified. The most prominent among them is galactose-and N-acetyl-d-galactosamine (Gal/GalNAc) lectin, a 260-kDa heterodimeric cell-surface protein consisting of a 170-kDa heavy chain (hgl) bound to a 35/31-kDa light chain (lgl) through disulfide linkage [19]. The light subunit is thought to attach to the membranes through glycosphingolipid anchors. The 260-kDa lectin is in complex with a 150-kDa intermediate subunit. The heavy chain has the carbohydrate recognition domain (CRD) displayed at the cell surface [20]. CRD recognizes target cells and ligand molecules through this domain. Overexpression of a mutant heavy chain subunit lacking essentially extracellular N-terminal domains (that is, mostly CRDs) conferred a dominant negative phenotype displaying reduced adherence and virulence in animal models [21]. Light subunits are also involved in adherence and virulence. Expression of mutated forms of lgl
(part of C-terminal deletion) showed a dominant negative phenotype. The heterodimeric complex of mutant lgl with hgl is formed, but this complex is functionally inactive [22]. The glycosyl-phosphatidylinositol (GPI) anchor of lgl may be important for the formation of a complex with hgl because an expression of C-terminal (GPI anchor region)-deleted molecules did not lead to a 260-kDa complex. In addition to Gal/GalNAc lectin, a number of other cell-surface molecules that are involved in adherence have been identified. Among these, Lysine and glutamic acid-rich protein 1 (KERP1) and cysteine protease adhesin (CPADH112) have been described in more detail [23].

Ca\(^{2+}\) also participates in the binding of ligands by Gal/GalNAc lectin [20]. CRD has a Ca\(^{2+}\) binding site, and in one study, it was shown that Ca\(^{2+}\) binding is required for interaction with the ligand [24]. In a more recent study, a Ca\(^{2+}\) binding site was identified, and a mutant that lost the ability to bind Ca\(^{2+}\) was generated. Though carbohydrate-binding function was retained by the mutant protein, the ability to agglutinate red blood cells (RBCs) was lost, suggesting that some properties of Gal/GalNAc lectin are modulated by Ca\(^{2+}\) ions [25]. The Ca\(^{2+}\)-binding chaperone protein calreticulin (CRT) is an E. histolytica cell-surface protein that binds complement component 1q (Clq) [26]. It participates in the phagocytosis of apoptotic immune cells, but not adherence or killing of normal cells such as Chinese hamster ovary (CHO) cells [26]. Moreover, the 2.15-Å X-ray structure of EhCRT showed a closed conformation of CRT with the dual carbohydrate and/or protein substrate-binding properties of lectin and that of chaperonin [27]. The pathway does not appear to be through Gal/GalNAc lectin and provides an alternate cell-surface–interacting system regulated by Ca\(^{2+}\).

**Cytolysis of target cells**

It has been shown very clearly that adherence of E. histolytica to target cells is required for subsequent cell lysis and tissue invasion [28–30]. Death of the target cells can be directly mediated through hydrolytic and toxin molecules of E. histolytica or through stimulation of apoptotic pathway initiated after contact with the parasitic cells [31]. Amebic cells encode and express a large number of different genes that have proteolytic activity [32]. Among these, cysteine proteinase 5 (Ehcp5) has gained attention because it is located on the cell surface and because of the absence of a functionally active homolog in the nonpathogenic species E. dispar [33]. Porin-like proteins of E. histolytica, amebapores, were also implicated in cytolysis carried out by amebic cells [34–36]. One of the consequences of the interaction of E. histolytica with target cells is a dramatic rise of Ca\(^{2+}\) levels in the latter after contact. Blocking target cell Ca\(^{2+}\) channels inhibited cell death. This is thought to be initiated by Gal/GalNAc lectin because the purified protein itself enhances Ca\(^{2+}\) levels in target cells [29,37]. However, the mechanism of target cell Ca\(^{2+}\) release on contact with E. histolytica is not clear.

A number of studies suggest that Ca\(^{2+}\) signaling is also involved in the ability of E. histolytica to initiate target cell killing. Blocking the rise of intracellular Ca\(^{2+}\) in the parasite prevents the initiation of the process of cytolysis [28,29,38,39]. Direct involvement of Ca\(^{2+}\) in amebic virulence was seen when a Ca\(^{2+}\)-binding transcription factor upstream regulatory element 3 binding protein (URE3BP) was found to regulate gene expression of virulence-associated genes. UREBP binds the promoter element of Gal/GalNAc lectin gene hgl5 [40,41]. It has 2 Ca\(^{2+}\)-binding EF-hand motifs and negatively regulates transcription in presence of Ca\(^{2+}\); that is, it binds the promoter DNA motif only in the absence of Ca\(^{2+}\) [40,42]. A Ca\(^{2+}\)-binding–defective mutant displayed a dominant positive phenotype, and cells expressing the mutant protein were more virulent [43]. URE3BP is likely to have a much wider role because a large fraction of amebic genes contain the URE3 motif recognized by URE3BP, thereby controlling expression of a number of genes [43]. URE3BP also shows an unusual localization at the
plasma membrane of trophozoites apart from that in the nucleus. Membrane association is regulated by a 22-kDa Ca\(^{2+}\)-dependent binding partner known as EhC2A [44]. Apart from these proteins, the ameba also displays a Ca\(^{2+}\)-dependent phospholipase activity that may have a role in virulence [45].

Phagocytosis and trogocytosis

Phagocytosis is intimately associated with the biology of *E. histolytica*. It displays a high rate of pinocytosis and phagocytosis that results in plasma membrane renewal every 30 min [46]. It phagocytoses a number of different cells that include RBCs, mammalian live and apoptotic cells, and bacterial cells. A number of reports have pointed out that phagocytosis plays a critical role in amebic virulence. Most of the evidence is based on the observed direct positive relationship of virulence potential with the phagocytic ability of an isolate. Generally, low phagocytic potential is correlated with less virulence. Moreover, a mutant defective in phagocytosis was found to be avirulent [47,48]. When this mutant was analyzed, it was observed that the level of EhCaBP1 was reduced several-fold in this mutant, suggesting that EhCaBP1 may be involved in phagocytosis [49]. Moreover, the essential role of Ca\(^{2+}\) in phagocytosis was also seen when chelation of Ca\(^{2+}\) in the cytoplasm led to inhibition of the process [50,51]. In the last few years, results from a number of studies have helped to outline a tentative molecular pathway of phagocytosis in *E. histolytica* [57]. It is clear from all these studies that Ca\(^{2+}\) plays a critical role from the initiation stage to the formation of phagosomes.

Phagocytosis is initiated by the recruitment of a C2-domain–containing protein kinase (EhC2PK) at the particle attachment site [52]. This recruitment requires Ca\(^{2+}\) and C2 domains and takes place when C2 binds membranes in the presence of Ca\(^{2+}\) [52,53]. We believe that the recruitment and enrichment of EhC2PK is the trigger for further assembly of the phagocytosis complex that starts with cups proceeding towards phagosomes. The formation of the phagocytosis complex requires multiple EhCaBPs, namely EhCaBP1, EhCaBP3, and EhCaBP5. EhC2PK recruits EhCaBP1 at the phagocytic stage, and Ca\(^{2+}\) is not required at this step. Once EhCaBP1 is at the phagocytic initiation site, it binds and recruits the atypical protein kinase EhAK1 in presence of Ca\(^{2+}\) [54,55]. EhAK1 is responsible for recruiting actin-related protein (Arp 2/3) complex proteins through the subunit EhARP1C [56]. Arp2/3 complex proteins in turn bind calmodulin-like CaBP EhCaBP3, and this step requires the presence of Ca\(^{2+}\) [57]. A typical calmodulin is thought to be absent in *E. histolytica* because no conserved gene has been seen in this system. EhCaBP3 is thought to be the closest homolog because it displays the highest degree of sequence similarity (about 49%) with calmodulins [58]. Both EhCaBP3 and EhCaBP5 bind atypical myosin 1B in the presence of Ca\(^{2+}\) [58,59]. Myosin 1B has been shown to be an important component of phagocytic machinery [46]. Imaging experiments have clearly shown that the myosin 1B–EhCaBP3 complex participates in the pseudopod fusion and subsequent separation from the membrane [58]. The role of EhCaBP5 in the context of its interaction with myosin 1B is not clear, though the results do indicate involvement in pseudopod fusion [59]. It is tempting to speculate that EhCaBP3 and EhCaBP5 regulate myosin 1B function and probably have different roles during phagocytosis [58,59]. Overexpression of Ca\(^{2+}\)–binding–defective mutants of all these proteins helped to delineate the participation of Ca\(^{2+}\) in different steps. Generally, these mutants display a dominant negative phenotype with respect to phagocytosis. Interestingly, overexpression of Ca\(^{2+}\)–binding–defective EhCaBP1 did not interfere with the formation of phagocytic cups or the process of recruitment, but the process of transition from cups to phagosomes was blocked, thereby indicating that EhCaBP1 recruitment is independent of Ca\(^{2+}\) [50]. Therefore, it appears that Ca\(^{2+}\) has both direct and indirect roles in the phagocytosis of *E. histolytica*. 
Trogocytosis has recently been shown to be a novel mechanism of target cell killing and virulence of *E. histolytica* [60,61]. The trophozoites tend to ingest fragments of live human target cells that lead to target cell death. This process has been termed amebic trogocytosis [61]. The process is likely to be initiated through the AGC family kinase 1 gene that is present only during the trogocytic event, but not during phagocytosis [62]. Subsequently, an EhC2PK-mediated pathway, similar to that observed for phagocytosis, is involved in the process [61]. Therefore, Ca$^{2+}$ also plays an important role in trogocytosis.

**Other CaBPs**

A number of as yet functionally uncharacterized CaBPs have been described in *E. histolytica*. The most prominent among these are 2 novel granule proteins grainin 1 and 2, which not only show a considerable structural similarity to EF-hand-motif–containing CaBPs but also bind Ca$^{2+}$ [63–
These proteins are thought to be involved in vesicular maturation and exocytosis. However, there is no evidence in support of these activities. Recent studies have suggested the involvement of grainin 2 in amebic virulence because it was found to be present differentially in virulent organisms [63]. EhCaBP2 displays 79% sequence identity with EhCaBP1 and also has 4 Ca\(^{2+}\)-binding EF-hand domains [66] (Fig 2). The central linker region between EF-hand domains 2 and 3 is most varied between EhCaBP1 and EhCaBP2 (Fig 2B). This region is thought to be involved in binding target molecules [66], suggesting that these 2 CaBPs are functionally different [67]. Unlike EhCaBP1, EhCaBP2 is involved in neither phagocytosis nor pseudopod formation. Moreover, these 2 proteins activate different sets of endogenous kinases and probably bind different sets of proteins in a Ca\(^{2+}\)-dependent manner [66–68]. However, the functional role of EhCaBP2 is yet to be deciphered. A nuclear-localized CaBP, EhCaBP6, was also characterized [69,70]. It was found to be involved in cell division by modulating microtubule dynamics by increasing the rate of tubulin polymerization through binding to E. histolytica beta-tubulin.

**Conclusion and future directions**

Calcium is known to be involved in many cellular processes in almost all eukaryotic systems. Therefore, it is not surprising that Ca\(^{2+}\) is also required for a number of processes, including...
pathogenesis in *E. histolytica*. The surprising part is the extensive participation of Ca\(^{2+}\) and CaBPs in a few systems such as phagocytosis not observed in any other eukaryotic systems (see Table 1). Moreover, direct involvement of CaBPs in regulating actin dynamics as shown in this organism is also quite unique. Evolution of this novel pathway regulating phagocytosis may be for adapting to a situation in which there is a very high rate of phagocytosis/endocytosis, leading to complete recycling of membrane every 30 min [71]. Analysis of live-cell imaging data does indicate that phagocytosis of a red blood cell is complete within 30 s after attachment. Ca\(^{2+}\) signaling is a rapid response, and mobilization of these EhCaBPs may not cause time delays after binding of a particle to the cell surface. Fast imaging of mobilization of different proteins during phagocytosis may help us to understand the nature of this rapid assembly process. The involvement of Ca\(^{2+}\) in many other amebic processes has not been investigated. Given the large number of CaBPs encoded by the *E. histolytica* genome, it will not be surprising to find Ca\(^{2+}\) signaling regulating a large number of pathways affecting the overall biology of *E. histolytica*.

References

1. Faust DM, Guillen N. Virulence and virulence factors in Entamoeba histolytica, the agent of human amoebiasis. Microbes Infect. 2012; 14: 1428–1441. https://doi.org/10.1016/j.micinf.2012.05.013 PMID: 22710276
2. Duggal P, Guo X, Haque R, Peterson KM, Ricklefs S, et al. A mutation in the leptin receptor is associated with Entamoeba histolytica infection in children. J Clin Invest. 2011; 121: 1191–1198. https://doi.org/10.1172/JCI45294 PMID: 21393862
3. Burgess SL, Petri WA Jr. The Intestinal Bacterial Microbiome and *E. histolytica* Infection. Curr Trop Med Rep. 2016; 3: 71–74. https://doi.org/10.1007/s40475-016-0083-1 PMID: 27525214
4. Iyer LR, Verma AK, Paul J, Bhattacharya A. Phagocytosis of Gut Bacteria by Entamoeba histolytica. Front Cell Infect Microbiol. 2019; 9: 34. https://doi.org/10.3389/fcimb.2019.00034 PMID: 30863724
5. Clapham DE. Calcium signaling. Cell. 2007; 131: 1047–1058. https://doi.org/10.1016/j.cell.2007.11.028 PMID: 18083096
6. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol. 2000; 1: 11–21. https://doi.org/10.1038/35036035 PMID: 11413485
7. Ghosh SK, Rosenthal B, Rogers R, Samuelson J. Vacular localization of an Entamoeba histolytica homologue of the plasma membrane ATPase (PMCA). Mol Biochem Parasitol. 2000; 108: 125–130. https://doi.org/10.1016/s0166-6851(00)00196-1 PMID: 10802325
8. Martinez-Higuera A, Salas-Casas A, Calixto-Galvez M, Chavez-Munguia B, Perez-Ishiwara DG, et al. Identification of calcium-transporting ATPases of Entamoeba histolytica and cellular localization of the putative SERCA. Exp Parasitol. 2013; 135: 79–86. https://doi.org/10.1016/j.exppara.2013.06.004 PMID: 23800535
9. Valle-Solis M, Bolanos J, Orozco E, Huerta M, Garcia-Rivera G, et al. A Calcium/Cation Exchanger Participates in the Programmed Cell Death and in vitro Virulence of Entamoeba histolytica. Front Cell Infect Microbiol. 2018; 8: 342. https://doi.org/10.3389/fcimb.2018.00342 PMID: 30327757
10. Rodriguez MA, Martinez-Higuera A, Valle-Solis MI, Hernandez-Alejandro M, Chavez-Munguia B, et al. A putative calcium-ATPase of the secretory pathway family may regulate calcium/manganese levels in the Golgi apparatus of Entamoeba histolytica. Parasitol Res. 2018; 117: 3381–3389. https://doi.org/10.1007/s00436-018-6030-4 PMID: 30084034
11. Raha S, Dalal B, Biswas S, Biswas BB. Myo-inositol trisphosphate-mediated calcium release from internal stores of Entamoeba histolytica. Mol Biochem Parasitol. 1994; 65: 63–71. https://doi.org/10.1016/0166-6851(94)90115-5 PMID: 7935629
12. Raha S, Giri B, Bhattacharyya B, Biswas BB. Inositol(1,3,4,5) tetrasphosphate plays an important role in calcium mobilization from Entamoeba histolytica. FEBS Lett. 1995; 362: 316–318. https://doi.org/10.1016/0014-5793(95)00265-b PMID: 7729520
13. Dominguez-Fernandez T, Rodriguez MA, Sanchez Monroy V, Gomez Garcia C, Medel O, et al. A Calpain-Like Protein Is Involved in the Execution Phase of Programmed Cell Death of Entamoeba histolytica. Front Cell Infect Microbiol. 2018; 8: 339. https://doi.org/10.3389/fcimb.2018.00339 PMID: 30319995
14. Monroy VS, Flores OM, Garcia CG, Maya YC, Fernandez TD, et al. Calpain-like: A Ca(2+) dependent cystein protease in Entamoeba histolytica cell death. Exp Parasitol. 2015; 159: 245–251. https://doi.org/10.1016/j.exppara.2015.10.005 PMID: 26496790
15. McLaughlin J, Muller M. A calcium regulated adenosine triphosphatase in Entamoeba histolytica. Mol Biochem Parasitol. 1981; 3: 369–379. https://doi.org/10.1016/0166-6851(81)90037-2 PMID: 6272107

16. Kobayashi S, Takeuchi T, Asami K, Fujiwara T. Entamoeba histolytica: ultrastructural localization of Ca2+-dependent nucleotidases. Exp Parasitol. 1982; 54: 202–212. https://doi.org/10.1016/0014-4894(82)90128-x PMID: 6182018

17. Bakker-Grunwald T, Parduhn H. The Ca(2+)-ATPase activity of Entamoeba histolytica is exposed towards the medium and towards the lumen of intracellular vesicles. Mol Biochem Parasitol. 1993; 57: 167–170. PMID: 8426610

18. Bhattacharya A, Padhan N, Jain R, Bhattacharya S. Calcium-binding proteins of Entamoeba histolytica. Arch Med Res. 2006; 37: 221–225. https://doi.org/10.1016/j.arcmed.2005.10.002 PMID: 16380322

19. Mann BJ. Structure and function of the Entamoeba histolytica Gal/GalNAc lectin. Int Rev Cytol. 2002; 216: 59–80. https://doi.org/10.1016/s0077-7696(02)16003-7 PMID: 12049210

20. Dodson JM, Lenkowski PW Jr., Eubanks AC, Jackson TF, Napodano J, et al. Infection and immunity mediated by the carbohydrate recognition domain of the Entamoeba histolytica Gal/GalNAc lectin. J Infect Dis. 1999; 179: 460–466. https://doi.org/10.1086/314610 PMID: 9878032

21. Vines RR, Ramakrishnan G, Rogers JB, Lockhart LA, Mann BJ, et al. Regulation of adherence and virulence by the Entamoeba histolytica lectin cystoplastic domain, which contains a beta2 integrin motif. Mol Biol Cell. 1998; 9: 2069–2079. https://doi.org/10.1090/mbc.9.8.2069 PMID: 9693367

22. Katz U, Anker S, Stolarsky T, Nuchamowitz Y, Mirelman D. Entamoeba histolytica expressing a dominant negative N-truncated light subunit of its gal-lectin are less virulent. Mol Biol Cell. 2002; 13: 4256–4265. https://doi.org/10.1090/mbc.E02-06-0344 PMID: 12475950

23. Daniela M. Faust NG. Cell-Surface Molecules as Virulence Determinants in Entamoeba histolytica. In Nozaki T, Bhattacharya A, editors. Amerbias. Tokyo: Springer Tokyo; 2014.

24. Chadee K, Johnson ML, Orozco E, Petri WA Jr., Ravdin JI. Binding and internalization of rat colonic mucins by the galactose/N-acetyl-D-galactosamine adherence lectin of Entamoeba histolytica. J Infect Dis. 1988; 158: 398–406. PMID: 2900266

25. Yadav R, Verma K, Chandra M, Mukherjee M, Datta S. Biophysical studies on calcium and carbohydrate binding to carbohydrate recognition domain of Gal/GalNAc lectin from Entamoeba histolytica: insights into host cell adhesion. J Biochem. 2016; 160: 177–186. https://doi.org/10.1093/jb/mvw024 PMID: 27008865

26. Vairithilingam A, Teixeira JE, Miller PJ, Heron BT, Huston CD. Entamoeba histolytica histolytic surface caitreliculin binds human c1q and functions in amebic phagocytosis of host cells. Infect Immun. 2012; 80: 2008–2018. https://doi.org/10.1128/IAI.06287-11 PMID: 22473608

27. Moreau C, Cioci G, Iannello M, Laffly E, Chouquet A, et al. Structures of parasite calreticulins provide insights into their flexibility and dual carbohydrate/peptide-binding properties. IUCrJ. 2016; 3: 408–419. https://doi.org/10.1107/S2052525216012847 PMID: 27840680

28. Ravdin JI, Sperelakis N, Guerrant RL. Effect of ion channel inhibitors on the cytopathogenicity of Entamoeba histolytica. J Infect Dis. 1982; 146: 335–340. https://doi.org/10.1093/infdis/i46.3.335 PMID: 6286794

29. Ravdin JI, Murphy CF, Guerrant RL, Long-Krug SA. Effect of antagonists of calcium and phospholipase A on the cytopathogenicity of Entamoeba histolytica. J Infect Dis. 1985; 152: 542–549. https://doi.org/10.1093/infdis/i52.3.542 PMID: 2863317

30. Ravdin JI, Guerrant RL. Role of adherence in cytopathogenic mechanisms of Entamoeba histolytica. Study with mammalian tissue culture cells and human erythrocytes. J Clin Invest. 1981; 68: 1305–1313. https://doi.org/10.1172/JCI110377 PMID: 6271810

31. Berninghausen O, Leippe M. Nercrosis versus apoptosis as the mechanism of target cell death induced by Entamoeba histolytica. Infect Immun. 1997; 65: 3615–3621. PMID: 9284127

32. Siqueira-Neto JL, Debnath A, McCall LJ, Bematche JA, Ndao M, et al. Cysteine proteases in protozoan parasites. PLoS Negl Trop Dis. 2018; 12(8): e0006512. https://doi.org/10.1371/journal.pntd.0006512 PMID: 30138453

33. Singh D, Naik SR, Naik S. Role of cysteine proteinase of Entamoeba histolytica in target cell death. Parasitol 2004; 129: 127–135. https://doi.org/10.1017/s0031182004005451 PMID: 15376772

34. Leippe M, Andra J, Nickel R, Tannich E, Muller-Eberhard HJ. Amebaporepeptide from cystoplastic granules of Entamoeba histolytica: isolation, primary structure, and pore formation in bacterial cytoplasmic membranes. Mol Microbiol. 1994; 14: 895–904. https://doi.org/10.1111/j.1365-2958.1994.tb01325.x PMID: 7715451

35. Bracha R, Nuchamowitz Y, Mirelman D. Transcriptional silencing of an amebapore gene in Entamoeba histolytica: molecular analysis and effect on pathogenicity. Eukaryot Cell. 2003; 2: 295–305. https://doi.org/10.1128/EC.2.2.295-305.2003 PMID: 12684379
36. Andra J, Leippe M. Pore-forming peptide of Entamoeba histolytica. Significance of positively charged amino acid residues for its mode of action. FEBS Lett. 1994; 354: 97–102. https://doi.org/10.1016/0014-5793(94)01103-6 PMID: 7525351

37. Ravdin JI, Moreau F, Sullivan JA, Petri WA Jr., Mandell GL. Relationship of free intracellular calcium to the cytolytic activity of Entamoeba histolytica. Infect Immun. 1988; 56: 1505–1512. PMID: 2897335

38. Aubott JN, Scarp A, Salata RA. Characterization of calcium channel forming activity of Entamoeba histolytica in model biological membranes. Lab Invest. 1991; 65: 518–524. PMID: 1661350

39. Makioke A, Kumagai M, Ohtomo H, Kobayashi S, Takeuchi T. Effect of calcium antagonists, calcium channel blockers and calmodulin inhibitors on the growth and encystation of Entamoeba histolytica and E. invadens. Parasitol Res. 2001; 87: 833–837. https://doi.org/10.1007/s004361004453 PMID: 11688889

40. Gilchrist CA, Holm CF, Hughes MA, Schraenman JM, Mann BJ, et al. Identification and characterization of an Entamoeba histolytica upstream regulatory element 3 sequence-specific DNA-binding protein containing EF-hand motifs. J Biol Chem. 2001; 276: 11838–11843. https://doi.org/10.1074/jbc.M00735200 PMID: 11278344

41. Gilchrist CA, Baba DJ, Zhang Y, Crasta O, Evans C, et al. Targets of the Entamoeba histolytica transcription factor URE3-BP. PLoS Negl Trop Dis. 2008; 2(8): e282. https://doi.org/10.1371/journal.pntd.0000282 PMID: 1884235

42. Gilchrist CA, Leo M, Line CG, Mann BJ, Petri WA Jr. Calcium modulates promoter occupancy by the Entamoeba histolytica Ca2+-binding transcription factor URE3-BP. J Biol Chem. 2003; 278: 4646–4653. https://doi.org/10.1074/jbc.M211271200 PMID: 12466263

43. Gilchrist CA, Moore ES, Zhang Y, Bousquet CB, Lannigan JA, et al. Regulation of Virulence of Entamoeba histolytica by the URE3-BP Transcription Factor. MBio. 2010; 1: e00057–10. https://doi.org/10.1128/mBio.00057-10 PMID: 20689746

44. Moreno H, Linford AS, Gilchrist CA, Petri WA Jr. Phospholipid-binding protein EhC2A mediates calcium-dependent translocation of transcription factor URE3-BP to the plasma membrane of Entamoeba histolytica. Eukaryot Cell. 2010; 9: 695–704. https://doi.org/10.1128/EC.00346-09 PMID: 20023071

45. Long-Krug SA, Fischer KJ, Hysmith RM, Ravdin JI. Phospholipase A enzymes of Entamoeba histolytica: description and subcellular localization. J Infect Dis. 1985; 152: 536–541. https://doi.org/10.1093/infdis/152.3.536 PMID: 2633316

46. Marion S, Laurent C, Guillen N. Signalization and cytoskeleton activity through myosin IB during the early steps of phagocytosis in Entamoeba histolytica: a proteomic approach. Cell Microbiol. 2005; 7: 1504–1518. https://doi.org/10.1111/j.1462-5822.2005.00573.x PMID: 16153248

47. Hirata KK, Que X, Melendez-Lopez SG, Debnath A, Myers S, et al. A phagocytosis mutant of Entamoeba histolytica is less virulent due to deficient protease expression and release. Exp Parasitol. 2007; 115: 192–199. https://doi.org/10.1016/j.exppara.2006.08.004 PMID: 16987516

48. Orozco E, Guanermos G, Martinez-Palomo A, Sanchez T. Entamoeba histolytica. Phagocytosis as a virulence factor. J Exp Med. 1983; 158: 1511–1521. https://doi.org/10.1084/jem.158.5.1511 PMID: 6313842

49. Sahoo N, Labruyere E, Bhattacharya S, Sen P, Guillen N, et al. Calcium binding protein 1 of the protozoan parasite Entamoeba histolytica interacts with actin and is involved in cytoskeleton dynamics. J Cell Sci. 2004; 117: 3625–3634. https://doi.org/10.1242/jcs.01198 PMID: 15252130

50. Jain R, Santi-Rocca J, Padhan N, Bhattacharya S, Guillen N, et al. Calcium-binding protein 1 of Entamoeba histolytica transiently associates with phagocytic cups in a calcium-independent manner. Cell Microbiol. 2008; 10: 1373–1389. https://doi.org/10.1111/j.1462-5822.2008.01314.x PMID: 18341598

51. Nunes P, Demaurex N. The role of calcium signaling in phagocytosis. J Leukoc Biol. 2010; 88: 57–68. https://doi.org/10.1189/jlb.0110028 PMID: 20400677

52. Somlata, Bhattacharya S, Bhattacharya A. A C2 domain protein kinase initiates phagocytosis in the protozoan parasite Entamoeba histolytica. Nat Commun. 2011; 2: 230. https://doi.org/10.1038/ncomms1199 PMID: 21407196

53. Somlata, Kamanna S, Agrahari M, Babuta M, Bhattacharya S, et al. Autophosphorylation of Ser428 of EhC2PK plays a critical role in regulating erythrophagocytosis in the parasite Entamoeba histolytica. J Biol Chem. 2012; 287: 10844–10852. https://doi.org/10.1074/jbc.M111.308874 PMID: 22753771

54. Mansuri MS, Babuta M, Ali MS, Bharadwaj R, Deep Jhang, G, et al. Autophosphorylation at Thr279 of Entamoeba histolytica atypical kinase EhAK1 is required for activity and regulation of erythrophagocytosis. Sci Rep. 2016; 6: 16963. https://doi.org/10.1038/srep16963 PMID: 26739245

55. Mansuri MS, Bhattacharya S, Bhattacharya A. A novel alpha kinase EhAK1 phosphorylates actin and regulates phagocytosis in Entamoeba histolytica. PLoS Pathog. 2014; 10(10): e1004411. https://doi.org/10.1371/journal.ppat.1004411 PMID: 25299184
56. Babuta M, Mansuri MS, Bhattacharya S, Bhattacharya A. The Entamoeba histolytica, Arp2/3 Complex Is Recruited to Phagocytic Cups through an Atypical Kinase EhAK1. PLoS Pathog. 2015; 11: e1005310. https://doi.org/10.1371/journal.ppat.1005310 PMID: 26646565

57. Babuta M, Kumar S, Gourinath S, Bhattacharya S, Bhattacharya A. Calcium-binding protein EhCaBP3 is recruited to the phagocytic complex of Entamoeba histolytica by interacting with Arp2/3 complex subunit 2. Cell Microbiol. 2018; 20: e12942. https://doi.org/10.1111/cmi.12942 PMID: 30133964

58. Aslam S, Bhattacharya S, Bhattacharya A. The Calmodulin-like calcium binding protein EhCaBP3 regulates phagocytosis and is involved in actin dynamics. PLoS Pathog. 2012; 8: e1003055. https://doi.org/10.1371/journal.ppat.1003055 PMID: 23300437

59. Kumar S, Aslam S, Mazumder M, Dahiya P, Murmu A, et al. The Calmodulin-like calcium binding protein EhCaBP3 of Entamoeba histolytica regulates phagocytosis and is involved in actin dynamics. PLoS Pathog. 2012; 8: e1003055. https://doi.org/10.1371/journal.ppat.1003055 PMID: 23300437

60. Ralston KS. Taking a bite: Amoebic trogocytosis in Entamoeba histolytica and beyond. Curr Opin Microbiol. 2015; 28: 26–35. https://doi.org/10.1016/j.mib.2015.07.009 PMID: 26277085

61. Ralston KS, Solga MD, Mackey-Lawrence NM, Somlata, Bhattacharya A, et al. Trogocytosis by Entamoeba histolytica contributes to cell killing and tissue invasion. Nature. 2014; 508: 526–530. https://doi.org/10.1038/nature13242 PMID: 24717428

62. Somlata, Nakada-Tsukui K, Nozaki T. AGC family kinase 1 participates in trogocytosis but not in phagocytosis in Entamoeba histolytica. Nat Commun. 2017; 8: 101. https://doi.org/10.1038/s41467-017-00199-y PMID: 28740237

63. Freitas MA, Alvarenga AC, Fernandes HC, Gil FF, Melo MN, et al. Differentially expressed genes of virulent and nonvirulent Entamoeba histolytica strains identified by suppression subtractive hybridization. Biomed Res Int. 2014; 2014: 285607. https://doi.org/10.1155/2014/285607 PMID: 25313356

64. Nickel R, Jacobs T, Urban B, Scholze H, Bruhn H, et al. Two novel calcium-binding proteins from cytoplasmic granules of the protozoan parasite Entamoeba histolytica. FEBS Lett. 2000; 486: 112–116. https://doi.org/10.1016/S0014-5793(00)02245-6 PMID: 11113449

65. Davis PH, Zhang X, Guo J, Townsend RR, Stanley SL Jr. Comparative proteomic analysis of two Entamoeba histolytica strains with different virulence phenotypes identifies peroxiredoxin as an important component of amoebic virulence. Mol Microbiol. 2006; 61: 1523–1532. https://doi.org/10.1111/j.1365-2958.2006.05344.x PMID: 16968225

66. Chakrabarty P, Sethi DK, Padhan N, Kaur KJ, Salunke DM, et al. Identification and characterization of EhCaBP2. A second member of the calcium-binding protein family of the protozoan parasite Entamoeba histolytica. J Biol Chem. 2004; 279: 12898–12908. https://doi.org/10.1074/jbc.M304716200 PMID: 14711825

67. Mustafi SM, Mutalik RB, Jain R, Chandra K, Bhattacharya A, et al. Structural characterization of a novel Ca2+-binding protein from Entamoeba histolytica: structural basis for the observed functional differences with its isoform. J Biol Inorg Chem. 2009; 14: 471–483. https://doi.org/10.1007/s00775-008-0463-7 PMID: 19137339

68. Gourinath S, Padhan N, Alam N, Bhattacharya A. Crystallization and preliminary crystallographic analysis of calcium-binding protein-2 from Entamoeba histolytica and its complexes with trionnyt and the IQ1 motif of myosin V. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2005; 61: 417–420. https://doi.org/10.1107/S1744309105007955 PMID: 16511057

69. Verma D, Murmu A, Gourinath S, Bhattacharya A, Chary KVR. Structure of Ca2+-binding protein-6 from Entamoeba histolytica and its involvement in trophozoite proliferation regulation. PLoS Pathog. 2017; 13: e1006332. https://doi.org/10.1371/journal.ppat.1006332 PMID: 28505197

70. Grewal JS, Padhan N, Aslam S, Bhattacharya A, Lohia A. The calcium binding protein EhCaBP6 is a microtubular-end binding protein in Entamoeba histolytica. Cell Microbiol. 2013; 15: 2020–2033. https://doi.org/10.1111/cmi.12167 PMID: 23848346

71. Aley SB, Cohn ZA, Scott WA. Endocytosis in Entamoeba histolytica. Evidence for a unique non-acidified compartment. J Exp Med. 1984; 160: 724–737. https://doi.org/10.1084/jem.160.3.724 PMID: 6206186

72. Kumar S, Padhan N, Alam N, Gourinath S. Crystal structure of calcium binding protein-1 from Entamoeba histolytica: a novel arrangement of EF hand motifs. Proteins. 2007; 68: 990–998. https://doi.org/10.1002/prot.21455 PMID: 17554780