General Introduction
1. The Pathogen *Entamoeba histolytica*

The enteric pathogen *Entamoeba histolytica* is responsible for human amoebiasis and has a global distribution with a high prevalence in the countries where poor socioeconomic and sanitary conditions predominate [1]. In 1828, James Annesley first hinted at an association of dysentery and liver abscess and Fedor Aldksandiovich Losch in 1873 first described *E. histolytica* in the context of amebiasis. Other historical landmarks in the study of *E. histolytica* and amoebiasis include the delineation of amoebic liver abscess and colitis by Osler and colleagues in 1890, its axenic culture by Diamond in 1961, differentiation of pathogenic (*E. histolytica* sensu strictu) from non-pathogenic (*E. dispar*) *E. histolytica* in 1979 [2]. Later on, in 1912 Leonard Rogers first designated emetine as an effective treatment for amoebiasis [3] followed by demonstration of the infective cyst form of *E. histolytica* by Walker and Sellards in 1913 [4]. The life cycle of *E. histolytica* was described by Dobel in 1925 and the morphological identity of *E. histolytica* and *E. dispar* and *E. histolytica* being pathogenic for human was demonstrated by Brumpt soon after [5].

The parasite is responsible for about 100000 deaths per annum, placing it second only to malaria in mortality due to protozoan parasites [6]. The human intestinal lumen is inhabited by at least eight distinct populations of amoeba namely *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. coli*, *E. hartmanni*, *E. polecki*, *Iodamoeba butschlii* and *Endolimax nana*. However except *E. histolytica* the rest are considered commensal organisms [7-11]. The disease is usually characterized by symptoms like abdominal pain and tenderness, painful sudden bowel evacuation (tenesmus), and diarrhea, developing over a period of one to several weeks often followed by weight loss. *E. histolytica* can spread in the bloodstream (haematogenously) after it has penetrated the colonic epithelium and can establish persistent extra-intestinal infections, most commonly amoebic liver abscess. Liver abscess is overwhelmingly the most common extra-intestinal manifestation of amoebiasis. *Entamoeba histolytica* has the taxonomic classification as in table 1.
2. Life cycle of *E. histolytica*

The life cycle of the parasite comprises two distinct morphological forms, the rapidly multiplying trophozoites and the dormant and infective cysts which measure about 10-15 μm in diameter and contain 1-4 nuclei. Chromatoid bodies are present in young cysts as elongated bars with bluntly rounded ends and the glycogen is often present as a concentrated mass. The trophozoites on the other hand possess a rapid unidirectional motility and measure about 60 μm in diameter. The infection is usually acquired by the ingestion of faecally contaminated food and water bearing amoebic cysts. Once inside the small intestine each cyst gives rise to eight trophozoites which then migrate from the small intestine to the large intestine [12]. Inside the large intestine the trophozoites adhere to the colonic mucus and the epithelial layers where they can either invade the colonic mucus leading to amoebic colitis or can migrate through the blood vessels to the extra-intestinal locations like liver, brain and lungs thereby causing fatal abscesses. Besides, within the large intestine some of the trophozoites get encysted again and form quadrinucleate cysts. Both the trophozoites and the cysts are then excreted.
along with the faeces. While the cysts survive for prolonged periods outside the host and possess the potential to establish new infections, the trophozoites survive only for a very short period and can not initiate a new infection.

Fig 1. Life cycle of *Entamoeba histolytica*  
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3. Pathogenesis of amoebic colitis

Adherence of the trophozoites to the colonic mucus

Adherence of the amoebic trophozoites to the colonic mucus is the key initial event in the pathogenesis of the disease which is mediated largely through the interaction of a D-galactose/N-acetyl-D-galactosamine (Gal/GalNAc)-specific amoebic lectin with host glycoconjugates [13, 14]. Lectin mediated adherence is also required for the cytolysis of host cells by amoeba since mammalian cells lacking N-terminal Gal and GalNAc protein modifications are resistant to amoebic killing [15, 16]. Besides lectin, there are several other proteins that also participate in the adherence process. These proteins include a 150 kDa intermediate weight lectin that co-purifies with Gal/GalNAc lectin, the serine rich *E. histolytica* protein (SREHP) and the 112 kDa adhesin that is composed of a cystein protease (EhCP112) and a protein with an adherence domain (EhADH112) [17-20].

Penetration of the colonic mucus barrier

Following adherence of the trophozoites to the mucus, *E. histolytica* cysteine proteases play the major role in the penetration of the host colonic mucus barrier by the trophozoites. Several studies have already established a direct correlation between the pathogenicity of different *E. histolytica* isolates with the expression of cysteine proteases [21, 22]. For instance clinical *E. histolytica* isolates release about 10-1000 fold more cysteine protease activity into the culture supernatants than the *E. dispers* isolates [23]. Moreover the trophozoite cysteine proteases can not only disrupt the colonic epithelium but also can induce an acute epithelial cell inflammatory response with the recruitment of neutrophils resulting in the immune mediated tissue damage [24]. All of these events ultimately lead to flask shaped ulceration.

Besides cysteine proteases the host cell lysis is also mediated by the pore forming secretory protein from the trophozoites. Amoebapore as it is called is a 5 kDa polypeptide bearing significant homology to granulysin and NK lysin produced by cytotoxic T cells and the natural killer (NK) cells [25].

*Entamoeba* induced host cell lysis has been shown to involve certain typical apoptotic cell death phenotypes like nuclear chromatin condensation and internucleosomal DNA fragmentation both in in vitro studies as well as in the animal
models of colitis and liver abscess [26, 27, 28]. Moreover the ability of the non-specific caspase inhibitors z-VAD-fmk to block the amoebic liver abscess formation in mice further emphasizes the apoptotic mode of cell death induction in the host cells [29]. In addition to direct cytolysis of host cells by amoebae, activation of an epithelial cell immune response by contact with and lysis of host cells, and subsequent recruitment of neutrophils, appears to be responsible for much of the tissue damage that occurs early during amoebic invasion [30]. The pre-IL-1β released by the lysed epithelial cells is processed to its active form by the amoebic cysteine proteases [31]. The paracrine action of IL-1β and cytolytically released IL-1α then stimulate production of additional inflammatory mediators including IL-8, IL-6, growth-related oncogene a (GRO-a, cyclooxygenase 2 (COX-2) and granulocyte–macrophage colony-stimulating factor (GM-CSF) by adjacent intestinal cells through a signaling pathway that is at least in part dependent on nuclear factor kB (NF-kB) [24, 32].

*E. histolytica* however evades the host immune response in several ways. Through the sequence similarity and the antigenic cross reactivity of Gal/GalNAc lectin with a human leukocyte antigen, CD59 the trophozoites prevent assembly of C5b-9 complement membrane attack complex formation and subsequent cell lysis [33]. Besides this, the glycosylphosphatidylinositol-anchored lipophosphoglycan / proteophosphoglycan molecules that coat amoebic trophozoites have also been proposed to serve as a barrier for complement [34, 35, 36]. Amoebic cysteine proteases also in addition to their role in invasion and activation of pre-IL-1β serve to protect amoeba from immune responses. For instance cysteine proteases rapidly degrade the complement anaphylatoxins C3a and C5a, secretory IgA and serum IgG as well as pre-IL-18 [37, 38, 39, 40]. *E. histolytica* trophozoites have also been found to suppress macrophage activation and major histocompatibility complex class II antigen presentation [41]. Figure 2 below depicts a model describing the stepwise invasion mechanism of the host colonic epithelia by the amoebic trophozoites.
Entamoeba histolytica adheres to colonic mucus via Gal/GalNAc lectin. Secreted amebic cysteine proteinases degrade colonic mucus

Amebic cysteine proteinases activate pre-IL-1-β in neighboring cells

Recruitment of neutrophils and other leukocytes contributes to tissue damage

Deep invasion of amebae with lateral spread and immune evasion results in flask-shaped ulcer with minimal inflammation

Fig 2. Model for the stepwise invasion of colonic mucosa by E. histolytica.
4. Genome of *E. histolytica*

*Genome structure*

The *E. histolytica* genome project was carried out by the Welcome Trust Sanger Institute, UK and the Institute for Genomic Research (TJGR), USA. The genome was predicted to code for about 9938 genes accounting for about 49% of the entire genome. Among these predicted genes 25% was found to contain introns while the rest are intronless. Moreover 31.8% of the predicted proteins were found to possess no homologues from the public databases [42]. The genome was also found to comprise about 25 types of long tandem arrays each containing between one and five tRNA types per repeat unit. However the parasite does not possess a mitochondrial genome emphasizing the lack of a functional mitochondrion.

*Nutrient metabolism*

The metabolism of *E. histolytica* is assumed to have been shaped by secondary gene loss and lateral gene transfer (LGT) primarily from bacterial lineages. Being a microaerophilic parasitic protozoan it actually lacks the enzymes for the tricarboxylic acid cycle and the mitochondrial electron transport chain. Glucose is used as the main energy source by the trophozoites and the glucose transporters used are more like the prokaryotic glucose/ribose porter family rather than the typical eukaryotic glucose transporters. When it comes to the question of the amino acid metabolism pathways the parasite genome was found to lack most of the enzymes involved in amino acid biosynthesis. However it still possesses the enzymes needed for the serine and cysteine biosynthesis which are absolutely essential for the production of the major intracellular thiol, the cysteine. The high levels of intracellular thiol actually compensates for the lack of glutathione and its associated enzymes which comprise a major component of the oxidative stress resistance in many organisms [43]. For nucleic acid biosynthesis *E. histolytica* relies on salvage pathways similar to *Giardia lamblia* and *Trichomonas vaginalis* [44]. The parasite genome completely lacks the enzymes needed for the de novo purine and the pyrimidine synthesis. Moreover like *G. lamblia*, *E. histolytica* also lacks ribonucleotide reductase [45]. Besides, *E. histolytica* is also unable to synthesize most of the fatty acids but retains the ability to synthesize most of the phospholipids. The organellar protein synthesis like in case of mitochondria and chloroplast is actually
dependent on folate. In *E. histolytica* however the genes coding for known folate
dependent enzymes and folate transporters are lacking probably on account of the
absence of the mitochondrial genome.

*Amoebic virulence determinants*

The *E. histolytica* genome has been found to code for a number of genes that
contribute towards the virulence of the pathogen. These virulence related genes include
the multi-subunit Gal/GalNAc lectins which are involved in the adhesion of the
trophozoites to the host colonic mucus, the cysteine proteases that degrade host
extracellular matrix and the pore forming peptides (amoebapores) capable of lysing target
cells [46]. Analysis of the genome reveals redundancy in the genes encoding these
virulence factors. Thirty homologues of the intermediate subunit and one homologue of
the heavy subunit of the GalGalNAc lectin were identified. Ten new cysteine proteinases
with predicted N-terminal transmembrane anchors, which might allow them to be
localized on the amoeba cell surface, were identified. In addition to three new
amoebapores a homologue of haemolysin III was identified, suggesting that, in addition
to amoebapores, haemolysins may have a role in host cell lysis.

*Vesicular transport machinery*

Although *E. histolytica* lacks morphologically identifiable rough endoplasmic
reticulum and Golgi apparatus [47], it encodes the basic components of the vesicular
transport machinery common to other eukaryotic cells with the coat complexes COPI,
COPII, clathrin and retromer all being present. Besides, the parasite genome also codes
for different members of Rab and Arf protein families that further complicates the vesicle
fusion and recycling steps associated with phagocytosis and pinocytosis in amoeba [48].
The parasite genome also provides significant evidences in support of a direct
communication between the regulators of the vesicle budding and cytoskeletal
rearrangement

*Signal transduction*

*E. histolytica* genome has been found to code for a huge number of different
signaling molecules that aid in the response of the trophozoite to different environmental
stimuli it encounters. This repertoire of signaling molecules is however predominated by
the kinases, 270 of which have been shown to be encoded within the genome and
represent members of all the seven families of eukaryotic protein kinase superfamily [49]. These include tyrosine kinases with SH2 domains, tyrosine kinase-like protein kinases and 90 putative receptor Ser/Thr kinases. The genome also codes for about 100 different protein phosphatases which regulate protein activities by dephosphorylation. However, one of the unique features of the amoebic phosphatases is the presence of leucine rich repeat (LRR) domains which are mainly involved in protein protein interactions, but were not previously been associated with phosphatases. Besides these the parasite genome also codes for numerous putative seven transmembrane receptors and trimeric G proteins which are probably involved in mediating autocrine stimulation of encystations [50]. Finally, *E. histolytica* has numerous cytosolic proteins involved in signal transduction, including Ras-family proteins, EF-hand calcium-binding proteins, phosphatidylinositol-3-OH kinase and MAP kinases. These represent the most varied set of signal-transduction-related proteins yet described in a single-celled eukaryote.

5. Stress response in *E. histolytica*

During its entire life cycle *E. histolytica* is exposed to many unfavourable environmental conditions. In some cases it protects itself through the formation of a resistant coat surrounding the parasite called the cyst while in other cases it triggers certain intracellular defense mechanisms.

**Oxidative stress**

*E. histolytica* is capable of tolerating only about 5% oxygen concentration in culture conditions [51]. But during the host tissue invasion the trophozoites are not only exposed to a highly aerobic environment but also to the reactive oxygen species (ROS) generated by the host’s protective effector cells like neutrophils and macrophages [52]. Moreover the toxicity of oxygen for the parasite is due to its ability to get transformed into ROS which can cause potential damage to the parasite through its interaction with essentially all the biomolecules present in the cell. Besides these oxygen can also damage some of the oxygen sensitive metabolic enzymes of the parasite like for instance pyruvate: ferredoxin oxidoreductase (PFOR) [53] that replaces the pyruvate dehydrogenase complex present in aerobic cells. PFOR actually intervenes in the oxidative decarboxylation of pyruvate to acetyl-coenzyme A which is further transformed.
into ethanol and acetate the final products of carbohydrate metabolism in the parasite. Since glycolysis is the main pathway through which the trophozoites generate ATP for cellular activities, PFOR inhibition by oxygen largely hampers the energy metabolism of the parasite. Thus to get rid of an excessive concentration of molecular oxygen the parasite reduces it to hydrogen peroxide through two distinct pathways. One of these pathways involves the initial reduction of oxygen to superoxide radical ($O_2^\cdot$) by an unidentified reductase [54] followed by Fe-superoxide dismutase (FeSOD) mediated reduction of $O_2^\cdot$ to hydrogen peroxide [55]. The other pathway however involves the direct reduction of molecular oxygen to hydrogen peroxide by NADPH:flavin oxidoreductase, Ehp34 [56]. Finally the hydrogen peroxide generated through both of these pathways is transformed into water by the action of peroxiredoxin (Eh29) [57], rubrerythrin [42] and/or thioredoxin reductase [58] enzymes.

**Thermal stress**

*E. histolytica* elicits a heat shock response as a defense against elevated temperature during its survival under different environments. An oligonucleotide based micro array analysis of 1131 unique genes from the parasite exposed to a temperature of 42°C for 4 h when compared to that of the parasite exposed to the optimum temperature of 37°C revealed a massive downregulation of 471 genes in response to the heat shock treatment [59]. These genes code for proteins involved in a variety of cellular activities like growth related processes, DNA metabolism and repair, fatty acid metabolism, RNA dynamics, signaling, cytoskeletal activities, vesicular trafficking, metabolic processes and virulence. Nonetheless a relatively small subset of analysed genes got upregulated in response to the heat shock treatment which includes the usual chaperones like three isoforms of HSP90, two isoforms of HSP70, DNAJ, protein containing a BAG domain, a p23 homologue, a candidate representing TPR domain and a protein with structural homology with CHIP (carboxyl terminus of Hsc70-interacting protein). Besides these the heat shock treatment of the trophozoites has also been found to activate ubiquitin and a component of 26S proteasome regulatory complex as well as several other ubiquitination components involved in the removal of damaged proteins. The trophozoites were also found to upregulate a copy of the Ehssp1 stress inducible gene [60] which has a possible role in the cellular adaptation to stress conditions.
6. The cytoskeleton of *E. histolytica*

A striking feature of *E. histolytica* trophozoites is their high motility manifested by fast locomotion, pleiotropism and continuous movement of intracellular components [61]. This intense motility of the amoebic trophozoites is supported by a highly dynamic cytoskeleton. The parasite genome indeed codes for several cytoskeletal proteins including actins, myosins and tubulins.

**Actins**

The trophozoite actins were first identified through immunostaining using heterologous antibodies raised against human and rabbit muscle actins [62, 63]. The amoebic actins showed the capacity to form filaments in vitro but unlike actins from other organisms lack the ability to bind to DNasel or polymerise at low temperatures [64, 65]. Moreover staining of the trophozoites with rhodamine phalloidin, a specific marker for polymerized actin revealed its localization and quantification in phagocytic cups formed during the phagocytosis of complete erythrocytes, pinocytic invaginations, uroids in the posterior parts of the cell and in the cortical regions of the cultured trophozoites [66, 67]. Further experiments showed that actin could be induced to organize complex structures such as adhesion plates and focal adhesions that can function as signal transduction organelles when trophozoites interacted with and degraded extracellular matrix proteins [68, 69]. However for performing many of these activities the amoebic actins need close association/interaction with a number of different cellular proteins. For instance, the organization of structures required for cellular movements, is accomplished by interaction between actin and multiple actin binding proteins. Some of these proteins connect actin filaments into extensive networks that can bind to other proteins in the cytoplasm or to the proteins and lipids in the plasmatic and vesicular membranes. While some others have a function in the nucleation and stabilization/destabilization cycles of the actin polymers [70]. The cellular activities like locomotion need dynamic turnover of actin which is actually mediated through the actions of two distinct classes of proteins, the profilin that sequesters actin monomers and thereby can inhibit or promote actin polymerization in a precise site within the cell and the cofillin and actin depolymerization factors (ADF) that facilitate actin filament depolymerisation [61]. Besides another
trophozoite protein, ABP-120 also plays significant role in regulating the polymerization/depolymerisation of amoebic actins.

**Myosins**

Myosins are proteins that act as molecular motors and move on actin filaments by making use of the chemical energy of ATP which is transformed into mechanical work. In contrast to the other eukaryotic cells which posses 18 different classes of myosins, *E. histolytica* genome codes for only two distinct classes [61]. Among these two groups those belonging to myosin II category are localized mostly in the uroid region of the trophozoites and at a very low density along the cell membranes and the frontal part of the cell. This specific localization of class II myosins indicates their probable involvement in cellular locomotion [71]. Later studies however reported myosin II together with actin as the components of ligand-receptor complexes or caps formed in the surface of the trophozoites [72].

The other class of myosin, myosin IB shows several similarities to other non-conventional myosins in higher eukaryotic cells [73]. This particular class of myosin has been found to interact with actin and α actinin. The structural analysis of these proteins reveals the presence of an ATP or GTP binding site, a putative domain for interaction with calmodulin and a SH3 domain to mediate interaction with cell membranes [61]. Overexpression of MyoIB indicated that this protein could be involved in phagocytosis, as a strain expressing three fold more myosin IB than the wild type strain was deficient in erythrocyte uptake, a characteristic feature of invasive trophozoites [74].

**Tubulins**

*E. histolytica* does not possess cytoplasmic microtubules but microtubules within dividing nuclei are present. This is supported by the fact that immunofluorescence experiments with antibodies raised against the different tubulins recognize them only in the nucleus [75, 76]. α, β and γ tubulins have been identified. α-tubulin shows nearly 50% sequence identity with those of other protozoa and human α-tubulin [77]. Amebic α-tubulin lacks the C-terminal polyacidic motif involved in polymerisation and binding to microtubule associated protein. A conserved tyrosine residue involved in microtubule stabilization is also missing. β-tubulin in *E. histolytica* shows greater than 50% identity with those from several organisms. Compared to α-tubulin gene which is present in
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multiple copies, β-tubulin is a single copy gene [77, 78]. γ-tubulin in eukaryotes have been found to be exclusively associated with microtubule organizing centre (MTOC). In *E. histolytica* MTOC has been detected as dense DNA containing matter with clusters of microtubules. Immunofluorescence experiments have revealed that γ-tubulin localizes in such clusters. Maximum expression of γ-tubulin occurs during S/G2 phase of the cell cycle. Possible disorganization of MTOC has been reported to occur when γ-tubulin had been blocked by RNAi techniques.

7. *E. histolytica* signal transduction mechanisms

**Integrin type mediated signaling**

During lytic stage of infection, *E. histolytica* trophozoites encounter a variety of cells and destroy them. Such a tissue invasion process in eukaryotes is characterized by local concentrations of actin along the cell membrane. These molecular junctions behave as attachment platforms and are decorated with integrin type receptors specially designed to interact with the ECM. Inside the cell the receptors interact with a host of molecules such as actin, talin, vinculin, α-actinin, paxillin, tensin, zyxin and focal adhesion kinase pp125FAK [79]. In *E. histolytica* two surface proteins bearing antigenic and functional similarities to β1 integrin have been identified, namely a 37 kDa and 140 kDa protein. Compounded to these, a multi-molecular surface hub consisting of phosphorylated paxillin, vinculin and pp125FAK has been described [80]. Blocking the 140 kDa surface protein with monoclonal antibody renders trophozoites incapable of adhesion to fibronectin and collagen.

Fibronectin (FN) binding triggers several signaling cascades. A surge in intracellular second messenger levels namely inositol tris phosphate (IP$_3$), diacyl glycerol (DAG), intracellular Ca$^{2+}$ and cyclic AMP. These members of the phosphoinositide and adenyl cyclase signaling pathways lead to the activation of PKC and PKA which subsequently activates an array of cytoskeleton associated proteins. PKC has been observed to migrate to the cytoskeleton-membrane fraction upon FN stimuli. Phorbol myristate acetate (PMA) binds to the cysteine rich C1 region of PKC and directly activates PKC and phosphorylation of downstream cytoskeletal proteins occur. U73122, a specific inhibitor of phospholipase C (PLC) suppressed the activation of PKC pathway.
Thus PLC acts as an upstream activator of PKC. FN stimulus also increases the plasma membrane adenyl cyclase level. Forskolin an activator of adenyl cyclase and membrane permeable dibutyryl cAMP (dcAMP) also increased cAMP levels and subsequently activated PKA. Certain bacterial toxins such as CTX from *Vibrio cholerae* and PTX from *Bordetella pertussis* also stimulate adenyl cyclase activity in *E. histolytica*. The increase in cAMP levels is largely accompanied by three effects namely upregulation of actin mRNA synthesis, shift in polymerised actin (F actin) to soluble actin (G actin) ratio and enhanced adhesion to fibronectin substrates.

**Rho mediated signaling**

Signaling pathways involving the participation of Rho family monomeric GTPases in actin organization have been extensively studied in higher eukaryotic cells. The first identified rho gene homologue in *E. histolytica* was cloned from parasite genomic DNA using PCR and degenerate oligonucleotide primers to GTP-binding sequences I and II that are conserved in ras family proteins. It was postulated that EhRho1 protein could participate as a factor controlling growth and differentiation [81]. EhRho1 is not a substrate for Rho-specific C3 exoenzyme, making it an unusual member of the Ras family. Since then, another six genes encoding proteins belonging to the Rho subfamily have been identified in *E. histolytica* that also lack the amino acid sequences to bind C3 exo enzyme and share 97% amino acid identity among them. Antibodies to recombinant EhRhoA1 indicated that Rho proteins are expressed in trophozoites and that activation by lysophosphatidic acid, possibly through a membrane receptor coupled to G<sub>α</sub>13 proteins, induces their participation in Rho signaling pathways leading to reorganization of actin and its interaction with myosin to form actomyosin structures. It has been suggested that actomyosin structures could have a role in functions requiring contractile force such as capping, phagocytosis of erythrocytes, chemotaxis, and adhesion to the substrate. Rac proteins have also been described in *E. histolytica* and their participation in actin organization has been inferred from experiments with mutants. Constitutively expressed activated Rac was found to affect phagocytosis and cap formation [82]. A Rho/Rac guanine nucleotide exchange factor (EhGEF) has been recently characterized as a protein with specificity for activation of Rac proteins, although it can also activate Rho1 to a certain extent. Overexpression of this activator
causes alterations in functions such as phagocytosis and chemotaxis. A kinase with homology to murine p21-activated kinase and yeast Ste20 was recently identified in *E. histolytica* (EhPAK). This protein can phosphorylate in vitro the regulatory chain of myosin II, myosin light chain kinase, and myosin 1B, suggesting a link with other signaling pathways that regulate myosin-actin interactions [83]. Presence of monomeric GTPases, their regulator proteins and diverse kinases in trophozoites in addition to the two main classical signaling routes suggest that regulation of actin organization is a complex process in which participation and collaboration between diverse signaling cascades is necessary.

**Ca**\(^{2+}\) regulation

The intracellular second messenger Ca\(^{2+}\) plays an important role in the execution of various cellular functions such as motility, secretion, cell division, differentiation and sodium and potassium dynamics [84]. Intracellular Ca\(^{2+}\) concentration is controlled by the uptake and release of Ca\(^{2+}\) across the plasma membrane and intracellular organelles by specific Ca\(^{2+}\) transporters. The largest reserve of intracellular Ca\(^{2+}\) is the endoplasmic reticulum. In *E. histolytica* a plasma membrane Ca\(^{2+}\) ATPase has been reported. Calmodulin (CaM), a classical Ca\(^{2+}\) binding protein, has been characterized. Elevation of internal Ca\(^{2+}\) levels is a prerequisite for processes such as motility and secretion. In *E. histolytica* trophozoites IP\(_3\) triggers the release of Ca\(^{2+}\) from intracellular stores [85]. Inositol 1,3,4,5 tetraphosphate (IP\(_4\)) was also shown to release Ca\(^{2+}\) from intracellular stores [86]. In addition to CaM, there are 26 putative calcium binding proteins in *E. histolytica*. These include the non-allelic variants of EhCaBP, EhCaBP1 and EhCaBP2. Both the genes are single copy and share 79% overall identity. EhCaBP1 has been found to bind actin. EhCaBP2 is a 15 kDa monomeric protein containing four canonical EF hand Ca\(^{2+}\) binding loops [87]. Both the EhCaBPs are phylogenetically close and it was postulated that they may have derived by a duplication event. Upon Ca\(^{2+}\) binding the proteins are predicted to undergo conformational change and the bound form then activates downstream proteins.
Transmembrane kinases

*E. histolytica* genome contains a huge repertoire of transmembrane kinases (TMK). There are 90 TMK genes reported in the parasite genome [42]. *E. histolytica* TMKs have been predicted to contain an N-terminal signal sequence, an extracellular domain and a single trans-membrane helix followed by a cytosolic kinase domain. Based on the predicted extracellular domains these kinases have been categorized into three different groups [42]. The first group consists of 50 members each of which posses CXXC rich repeats in the extracellular domains. The other two groups either carry cysteine rich domains containing CXC repeats or lack the cysteine rich extracellular domains. Each of these groups however is subdivided into different categories depending upon the sequence features of the sub-domain VII within the kinase domain [88]. Among these different categories, the B family members posses the signature KLTDGS in sub-domain VII and can be further classified into three families (B1, B2 and B3) based on the size of the protein and the distribution of CXXCXXGY motifs within the extracellular domain. The B1 family TMKs has been suggested to be associated with the proliferation of the parasite under different environmental conditions [88]. Besides, there are a few reports on the functional implications of some specific TMKs. The phagosome-associated TMK96 (PATMK) for instance has been found to be involved in erythrophagocytosis [89]. EhTMKB1-9, a member of the B1 family TMK has been shown to be a serum induced TMK and a probable role of the kinase in cellular proliferation and virulence has also been suggested [90]. The expression of a truncated TMK39 and TMK54 however has been shown to interfere with the phagocytosis of the apoptotic lymphocytes by the trophozoites and the growth of the amoeba respectively [91]. The functional implications of the rest of the TMKs are relatively unknown till date. Moreover the different TMK families of the parasite represent functionally different receptors in terms of sensing a variety of extracellular signals thereby relaying them to a number of downstream signaling pathways.
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