Microreview

Cell signalling and *Trypanosoma cruzi* invasion

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Summary

Mammalian cell invasion by the protozoan pathogen *Trypanosoma cruzi* is critical to its survival in the host. To promote its entry into a wide variety of non-professional phagocytic cells, infective trypomastigotes exploit an arsenal of heterogenous surface glycoproteins, secreted proteases and signalling agonists to actively manipulate multiple host cell signalling pathways. Signals initiated in the parasite upon contact with mammalian cells also function as critical regulators of the invasion process. Whereas the full spectrum of cellular responses modulated by *T. cruzi* is not yet known, mounting evidence suggests that these pathways impinge on a number of cellular processes, in particular the ubiquitous wound-repair mechanism exploited for lysosome-mediated parasite entry. Furthermore, differential engagement of host cell signalling pathways in a cell type-specific manner and modulation of host cell gene expression by *T. cruzi* are becoming recognized as essential determinants of infectivity and intracellular survival by this pathogen.

Introduction

The intracellular protozoan pathogen *Trypanosoma cruzi* causes Chagas' disease in humans, a chronic inflammatory condition characterized by cardiomyopathy and digestive disorders (for recent reviews: Chimelli and Scaravilli, 1997; Zhang and Tarleton, 1999; Engman and Leon, 2002). Naturally acquired *T. cruzi* infections are initiated in the dermal layers or conjunctival mucosa by infective metacyclic trypomastigotes transmitted by an infected haematophagous triatomine vector such as *Triatoma infestans*. Completion of the intracellular replicative

cycle takes 3–5 days, after which infective trypomastigotes, released into the bloodstream of the host, are disseminated throughout the body and infect tissues at distal sites. Thus, the ability to infect and replicate within a variety of cell types is an essential feature of the *T. cruzi* life cycle in the mammalian host. Whereas parasites are eventually cleared from most tissues and parasitaemia is no longer detectable in the post-acute stages of infection, low-level persistence, particularly in the myocardium and smooth muscle, contributes to the pathology associated with chronic Chagas' disease. Presently, several determinants of host cell invasion have been uncovered, but the molecular requirements for intracellular growth and parasite persistence remain poorly understood. The resulting complex interplay between *T. cruzi*-dependent modulation of the host cell environment and immune avoidance strategies is likely to influence the outcome of infection.

Elucidation of the molecular components regulating establishment of *T. cruzi* infection is critical to understanding the pathogenesis of Chagas' disease. Predictably, communication between pathogen and host cell directs the complex *T. cruzi* infection process at multiple stages. *In vitro* models of infection (invasion and intracellular replication) have been very useful for studying the molecular and cellular basis of these interactions. Whereas early studies concentrated primarily on the role of *T. cruzi* trypomastigote surface antigens in mediating attachment of parasites to non-professional phagocytic cells (reviewed by Frasch, 1994; 2000; Burleigh and Andrews, 1995a), a more recent focus has been the role of signal transduction pathways in co-ordinating *T. cruzi* invasion (Burleigh and Andrews, 1998) and modulating host cell gene expression (Chuenkova *et al.,* 2001; Vaena de Avalos *et al.,* 2002). A variety of cell surface receptors, secondary messengers and transcription factors from different pathways appear to have been exploited by the parasite to mediate and affect parasite invasion and survival. The best characterized of such signalling events related to host cell invasion is exemplified by Ca^{2+}-signalling elicited in mammalian host cells by infective trypomastigotes. In particular, Ca^{2+}-dependent fusion of lysosomes with the host cell plasma membrane has emerged as an important route for parasite entry. These pathways will be discussed in detail below. Recent studies also suggest hat interaction with mammalian host cells triggers contact-dependent sig-

nalling in protozoan parasites as well, a phenomenon that appears to be universal among invasive eukaryotic pathogens (Moreno *et al.*, 1994; Shaw, 1995; Lu *et al.*, 1997; Pezzella *et al.*, 1997; Carruthers and Sibley, 1999; Carruthers *et al.*, 1999; Vieira and Moreno, 2000).

Trypanosoma cruzi exploits the mammalian cellular wound-repair pathway for invasion

Trypomastigotes, the mammalian infective forms of *T. cruzi*, are relatively large (~20 μm in length), motile organisms that have the capacity to infect most nucleated cell types. Non-dividing trypomastigotes must establish residence within the host cell cytoplasm and differentiate into amastigotes before undergoing the first division in a process that takes ~24 h to complete (illustrated in Fig. 1A). Following attachment to the host cell membrane, signals triggered by the parasite initiate the gradual entry process into a host cell membrane-derived vacuole which is accomplished within 10 min (Rodríguez *et al.*, 1996). Unlike many intracellular pathogens that adeptly avoid fusion with host cell lysosomes (reviewed by Sinai and Joiner, 1997; Amer and Swanson, 2002), transient residence within an acidic lysosomal compartment is a prerequisite for *T. cruzi* survival (Ley *et al.*, 1990). Exposure of trypomastigotes to the acidic environment of the lyso-

somal vacuole is required for the activity of the secreted pore-forming *T. cruzi* molecule, TcTox, which aids in the disruption of the vacuole (Andrews and Whitlow, 1989; Andrews *et al.*, 1990). Moreover, low pH is thought to be an important trigger for trypomastigote to amastigote differentiation (Tomlinson *et al.*, 1995), a process that is initiated in the vacuole and completed in the cytoplasm (Fig. 1A). Additional environmental cues required for completion of the intracellular life cycle of this parasite may also be provided by the lysosome-derived vacuole.

Trypanosoma cruzi trypomastigotes have evolved the unique capacity to harness the cellular wound-repair mechanism, involving Ca^{2+}-regulated lysosome exocytosis (Rodríguez *et al.*, 1997; Reddy *et al.*, 2001), as the basis for entry into the host cell and for the formation of the short-lived parasitophorous vacuole (Tardieux *et al.*, 1992) (Fig. 1A and B). Initiated by *T. cruzi*-induced signals, host cell lysosomes are recruited to the plasma membrane along microtubules in a kinesin-dependent manner (Tardieux *et al.*, 1992; Rodríguez *et al.*, 1996), where targeted fusion at the parasite attachment site is regulated by the lysosome-associated Ca^{2+}-sensor synaptotagmin VII (Martinez *et al.,* 2000; Reddy *et al.,* 2001). In marked contrast to actin-dependent processes such as phagocytosis or membrane-ruffling utilized by many invasive microorganisms for internalization (Reed *et al.*, 1996;
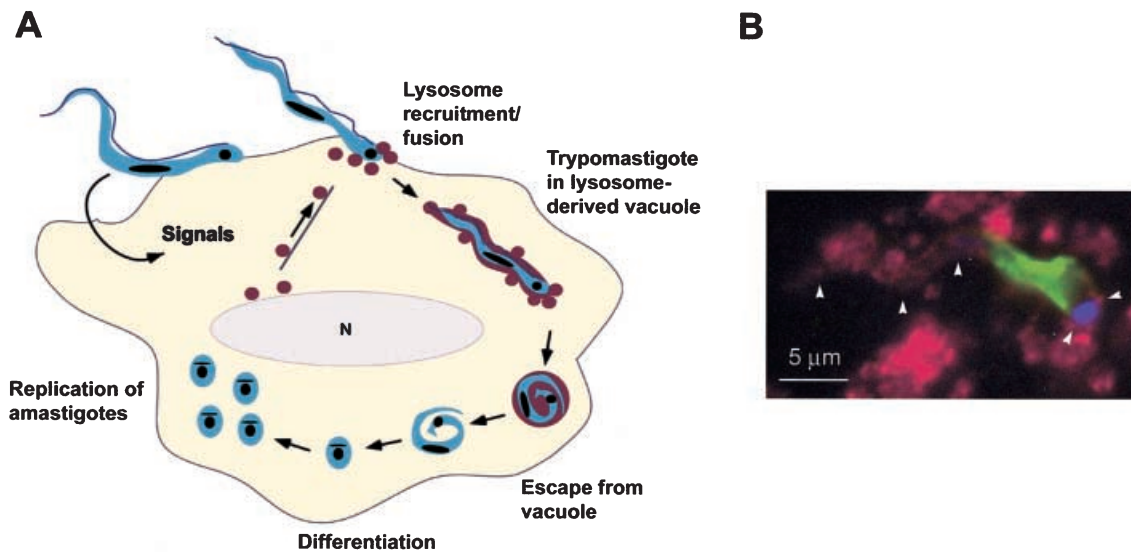

Fig. 1. Invasion by *T. cruzi* involves early recruitment of host cell lysosomes.
A. Schematic representation of the *T. cruzi* intracellular infection cycle: trypomastigotes associate with the surface of mammalian cells, trigger signalling pathways that lead to the localized recruitment and fusion of host cell lysosomes with the plasma membrane and generation of a lysosome-derived parasitophorous vacuole. Parasites are released into the cytoplasm following disruption of the vacuole, differentiate to replicative amastigote forms and begin to divide at ~24 h post infection.
B. Immunofluorescence staining demonstrating co-localization of host cell lamp-1 (red), a lysosomal marker, with partially internalized *T. cruzi* trypomastigote (extracellular portion stained green) in a primary rat cardiomyocyte.

Sansonetti *et al.*, 1999; Zhou *et al.*, 1999; Edwards *et al.*, 2000), depolymerization of host cell actin microfilaments enhances the Ca^{2+}-regulated lysosome-mediated *T. cruzi* entry pathway (Rodríguez *et al.*, 1997).

Mechanisms of *T. cruzi*-induced Ca^{2+}-signalling

Prior to entry, invasive *T. cruzi* trypomastigotes elicit asynchronous and repetitive intracellular $[Ca^{2+}]_i$-transients in mammalian cells (Moreno *et al.*, 1994; Tardieux *et al.*, 1994; Caler *et al.*, 2000; Scharfstein *et al.*, 2000). Such changes in intracellular calcium levels regulate parasite invasion by promoting transient actin rearrangements (Rodríguez *et al.*, 1995) and lysosome-plasma membrane fusion (Rodríguez *et al.*, 1997). The $[Ca^{2+}]_i$-transients are initiated relatively slowly (1–2 min post addition) in response to live trypomastigotes (Moreno *et al.*, 1994; Tardieux *et al.*, 1994; Caler *et al.*, 2000), suggesting that proximity to the host cell is a prerequisite for induction of Ca^{2+}-signalling by this pathogen (Caler *et al.*, 2000; Scharfstein *et al.*, 2000). In support of this, constitutively released/secreted trypomastigote components fail to generate an appreciable Ca^{2+}-response in mammalian cells (Burleigh and Andrews, 1995b). Following an initial increase in $[Ca^{2+}]_i$ in infected and neighbouring uninfected cells in culture, $[Ca^{2+}]_i$ levels decline at later times of infection (3–5 days) (Low *et al.*, 1992) indicating that cellular Ca^{2+} homeostasis is disrupted in *T. cruzi*-infected mammalian cells for the duration of the intracellular infection cycle. Presently, our understanding of the mechanism(s) by which infective *T. cruzi* trypomastigotes induce Ca^{2+}-signalling in mammalian cells is far from complete. Highlighted below are three distinct models for parasite-dependent Ca^{2+}-signalling that have been proposed by different groups. Although the data to support each model ostensibly excludes the other two, these controversial reports may be indicative of a more complex set of interactions involving redundant mechanisms for activation of this important signalling pathway.

A tale of two peptidases

Two peptidase-dependent mechanisms for *T. cruzi*-induced Ca^{2+}-signalling in mammalian cells have been described. One involves the serine endopeptidase, oligopeptidase B (Burleigh and Andrews, 1995b; Burleigh *et al.*, 1997; Caler *et al.*, 1998) and the other requires the participation of the secreted/lysosomal cysteine protease, cruzipain (Scharfstein *et al.*, 2000). Whereas current evidence suggests that these are discrete mechanisms, the following similarities emerge when comparing the two models: peptidase-dependent cleavage of inactive precursors yield active Ca^{2+} agonists that bind to G-protein-coupled receptors and trigger mobilization of intracellular

Ca^{2+} stores in an IP3-mediated manner (Fig. 2, pathways 2 and 3). In the case of oligopeptidase B-dependent signalling, peptide processing is postulated to occur at an intracellular location (oligopeptidase B is cytosolic) before release of the active parasite-derived agonist (Burleigh *et al.*, 1997; Caler *et al.*, 1998). Cruzipain, on the other hand, is secreted and its substrate is of host origin (Scharfstein *et al.*, 2000). In addition to oligopeptidase B and cruzipain-dependent models of host cell Ca^{2+} signalling, peptidase-dependent extracellular matrix remodelling and localized signalling events are emerging as key regulators of *T. cruzi* pathogenesis. For example, recent studies have demonstrated that a parasite-derived 80 kDa prolyl endopeptidase with collagen I degrading activity facilitates invasion of mammalian cells (Santana *et al.*, 1997; Grellier *et al.*, 2001), an activity that is likely to be highly relevant *in vivo* during the navigation of interstitial tissue spaces by trypomastigotes.

I. Oligopeptidase B-dependent Ca^{2+}-signalling

Trypanosoma cruzi trypomastigotes express a soluble Ca^{2+}-signalling activity (Tardieux *et al.*, 1994; Burleigh and Andrews, 1995b) that activates host cell phospholipase C (PLC) to promote the generation of inositol triphosphate (IP3) and mobilization of Ca^{2+} from thapsigargin-sensitive intracellular stores (Rodríguez *et al.*, 1995). This activity was shown to be coupled to the activity of an 80 kDa cytosolic serine peptidase denominated oligopeptidase B (Opb) (Burleigh *et al.*, 1997). However, neither isolated native nor recombinant peptidase were capable of triggering $[Ca^{2+}]_i$-transients in mammalian cells. Given its homology to prohormone processing enzymes (Fuller *et al.*, 1988; Kreil, 1990), it was proposed that Opb might function to generate a $[Ca^{2+}]_i$-agonist from an inactive precursor peptide (Burleigh *et al.*, 1997) (Fig. 2, pathway 2). Several pieces of evidence support the hypothesis that *T. cruzi* Opb functions indirectly to induce $[Ca^{2+}]_i$-transients during *T. cruzi* invasion. First, specific Opb-blocking antibodies inhibited the soluble Ca^{2+}-signalling activity (Burleigh *et al.*, 1997). Second, oligopeptidase B null mutants (*opb–/–*) generated by targeted gene disruption were significantly impaired with respect to their ability to infect mammalian cells *in vitro* and to establish infections in mice (Caler *et al.*, 1998). Furthermore, residual host cell invasion by *opb–/–* mutants is insensitive to thapsigargin or pertussis-toxin pretreatment of mammalian cells, thereby implicating oligopeptidase B in the IP_3-mediated Ca^{2+}-dependent entry pathway (Caler *et al.*, 1998; 2000). Further evidence was provided by biochemical reconstitution experiments in which purified recombinant Opb restored Ca^{2+}-signalling activity in soluble extracts prepared from *opb–/–* trypomastigotes (Caler *et al.*, 1998). Whereas biochemical studies and gene knockout experi-
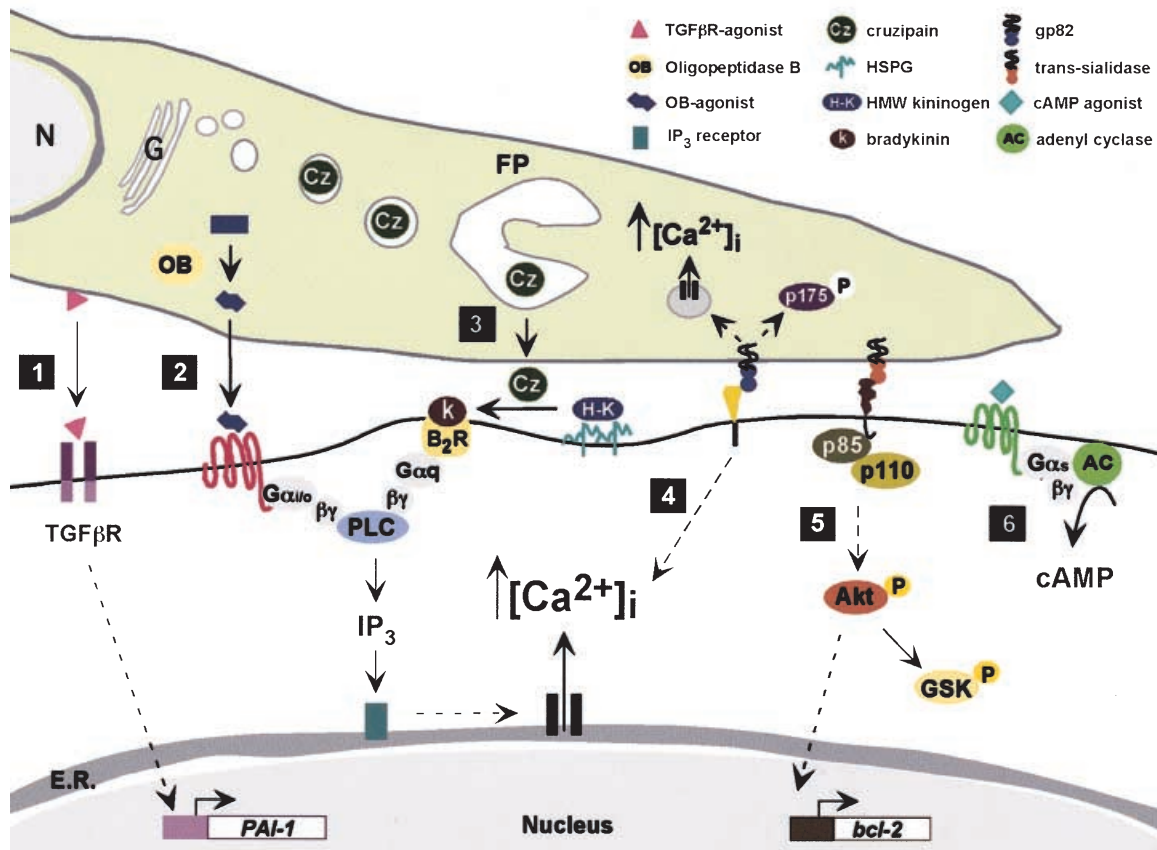
Fig. 2. Schematic diagram summarizing molecular components of signalling pathways triggered in non-professional phagocytic cells by infective T. cruzi trypomastigotes. (1) Secreted/released trypomastigote molecule(s) stimulate TGF-β receptors in epithelial cells and activate transcription of TGF-β-responsive genes such as plasminogen activator inhibitor-1 (PAI-1). (2) Cytosolic *T. cruzi* oligopeptidase B cleaves an inactive precursor to generate an active Ca^{2+}-agonist that is released from the parasite and binds a pertussis-toxin sensitive G-protein-coupled receptor. This stimulates phospholipase C (PLC) activity to generate inositol, 3,4,5 triphosphate (IP3) which binds its receptor and promotes release of Ca^{2+} from intracellular stores such as the endoplasmic reticulum (ER). (3) Cruzipain (Cz) secreted from the flagellar pocket (FP) of *T. cruzi* cleaves host high molecular weight kininogen (H-K) to generate short-lived kinins (k) which bind to the bradykinin receptor (B_2K) to stimulate IP3-mediated Ca^{2+} release. (4) Bi-directional Ca^{2+}-signalling mediated by metacyclic surface glycoprotein, gp82 binding to unknown host cell receptor. (5) *Trans*-sialidase (TS)-mediated stimulation of host cell PI-3-kinase (p85/p110) signalling, activation of downstream serine/threonine kinase Akt, phosphorylation of glycogen synthase kinase (GSK) and activation of *bcl-2* transcription. (6) Stimulation of host cell adenyl cyclase (AC) by a *T. cruzi* cAMP agonist leads to cAMP elevation in the host cell.

ments highlight the role for oligopeptidase B in *T. cruzi* invasion, a critical test of this phenotype, reintroduction of functional oligopeptidase B gene in the *opb*–/– mutants has not been reported (Caler *et al.*, 1998). Nevertheless, these studies demonstrate, importantly, that residual Ca^{2+}-signalling activity is associated with live *opb*–/– trypomastigotes (Caler *et al.,* 2000) and is present in soluble extracts prepared from *opb*–/– parasites (Caler *et al.*, 1998), indicating redundant pathways for generation of $[Ca^{2+}]_i$-transients in mammalian cells.

II. Cruzipain-dependent Ca^{2+}-signalling

Recently, Scharfstein and colleagues reported that *T. cruzi* invasion of a subset of mammalian cells is facilitated by $[Ca^{2+}]_i$-transients initiated through host cell bradykinin

(B_2R) receptors by short-lived kinins (Scharfstein *et al.,* 2000) (Fig. 2, pathway 3). Previous work from this group had demonstrated that active kinins, such as bradykinin, could be liberated from host kininogen by a constitutively secreted parasite cysteine protease, cruzipain (Del Nery *et al.*, 1997). Incubation with purified exogenous cruzipain promoted $[Ca^{2+}]_i$-transients in CHO cells expressing heterologous B_2R receptors but not in wild-type CHO cells (Scharfstein *et al.,* 2000). Furthermore, pretreatment of cells with cruzipain or bradykinin resulted in enhanced host cell invasion by *T. cruzi* and B_2R-dependent entry could be effectively blocked with membrane-permeable cysteine protease inhibitors but not with hydrophilic compounds. Together, these results suggest that protease-dependent kinin generation follows localized secretion of cruzipain at the site of intimate contact between *T.*

cruzi trypomastigotes and host cell (Scharfstein *et al.*, 2000). Interestingly, cruzipain-dependent liberation of kinins from host kininogen was greatly enhanced in the presence of heparan sulphate (Lima *et al.,* 2002), a molecule with recognized importance in facilitating *T. cruzi* invasion of non-professional phagocytic cells (Herrera *et al.*, 1994).

Bi-directional Ca^{2+}-signalling mediated by T. cruzi *metacyclic surface glycoprotein (gp82)*

Metacyclic trypomastigotes express several stage-specific surface glycoconjugates, encoded by multigene families (Araya *et al.*, 1994; Frasch, 2000), that have been implicated in host cell recognition and invasion (Teixeira and Yoshida, 1986; Araguth *et al.*, 1988; Yoshida *et al.*, 1989). These include gp82, gp90 and gp35/50. Induction of Ca^{2+}-transients in HeLa or Vero cells in response to metacyclic extracts was attributed predominantly to gp82 (Dorta *et al.*, 1995; Ruiz *et al.*, 1998). In contrast, levels of endogenous gp90 and gp35/50 expression inversely correlated with the ability of different *T. cruzi* strains to invade non-professional phagocytic cells (Yoshida *et al.*, 1997; Ruiz *et al.*, 1998; Malaga and Yoshida, 2001). Thus, interaction of these ligands with their putative receptors appears to interfere, directly or indirectly, with the ability of gp2 to engage its receptor(s) and trigger Ca^{2+}-signalling. Whereas the significance of such competition is not yet understood, the relative expression of these parasite surface molecules may be an important determinant of tissue tropism.

  Induction of $[Ca^{2+}]$-transients during early parasite–host cell interactions is not limited to the host cell. Single cell measurements of $[Ca^{2+}]_i$ carried out in Fura2-AM-loaded *T. cruzi* trypomastigotes revealed that host cell attachment coincides with increased levels of $[Ca^{2+}]_i$ in the parasite (Moreno *et al.*, 1994). Similar to host cell Ca^{2+}-signalling, elevation of parasite intracellular $[Ca^{2+}]$ was found to be required for efficient invasion (Moreno *et al.*, 1994; Yakubu *et al.*, 1994). Cross-linking gp82 on the surface of metacyclic trypomastigotes with specific mAbs was shown to be sufficient to produce elevated $[Ca^{2+}]_i$ levels in these parasites, an effect that was mimicked with mammalian cell extracts (Ruiz *et al.*, 1998). Evidence suggests that the gp82-mediated increase in parasite intracellular $[Ca^{2+}]_i$ is dependent upon activation of phospholipase C (Yoshida *et al.,* 2000) downstream of protein tyrosine kinase activation, which results in the phosphorylation of a 175 kDa *T. cruzi* protein (Favoreto *et al.*, 1998). Thus the model for gp82-dependent signalling assigns a pivotal role to this parasite surface glycoprotein in mediating cross-talk between infective metacyclic trypomastigotes and mammalian host cells (Fig. 2, pathway 4). The observation that intratrypomastigote $[Ca^{2+}]_i$ is sustained at an elevated level for several hours after host cell entry (Moreno *et al.*, 1994) also suggests that Ca^{2+}-signalling contributes to events in the intracellular parasite life cycle.

Redundancy or discrepancy?

In summary, three non-overlapping mechanisms have been presented which account for initiation of host cell Ca^{2+}-transients by infective *T. cruzi* trypomastigotes. Presently, the inter-relationship between and relative contribution of the proposed mechanisms of *T. cruzi*-induced Ca^{2+}-signalling toward the host cell invasion process is unclear. Apparent contradictions between these models remain an unresolved issue in the field. For example, studies describing oligopeptidase B-dependent Ca^{2+}-signalling exclude the participation of cruzipain because the signalling activity was insensitive to cysteine proteases despite the presence of measurable cysteine protease activity in these parasite extracts (Burleigh and Andrews, 1995b; Caler *et al.*, 1998). Similarly, inhibitors of oligopeptidase B (e.g. leupeptin) failed to block the soluble activity attributed to gp82 in other studies (Dorta *et al.*, 1995). Whereas certain inconsistencies might be explained by differences in host cell type, *T. cruzi* strain, or life cycle stage, others are likely to reflect differences in methodology. For example, in some experiments (Dorta *et al.*, 1995) measurement of intracellular $[Ca^{2+}]$ was conducted using normally adherent cell types (HeLa and Vero) in suspension. Because adherence-dependent Ca^{2+}-signalling has been clearly demonstrated in mammalian cells (Arora *et al.*, 1995; Bernardo *et al.*, 1997), it is possible that the context in which these experiments were carried out (Dorta *et al.*, 1995) precluded detection of the peptidase-dependent activities observed in the studies that employed adherent cells (Caler *et al.*, 1998; Scharfstein *et al.,* 2000). Thus, while these seemingly disparate models warrant further examination, it is certainly reasonable to expect redundancy at the level of host cell Ca^{2+}-signalling especially given the importance of this pathway in cell invasion by this pathogen.

Other host cell signalling pathways

Significant experimental evidence has accumulated that point to the involvement of various host cell signalling pathways in *T. cruzi* invasion of host cells and for intracellular survival. In addition to facilitating lysosome-mediated entry, some of these pathways appear to additionally induce anti-apoptotic mechanisms and effect immune modulations. Cross-talk between these pathways is likely to exist and selective utilization of these pathways is expected depending on the type of host cell involved.

Cyclic AMP

Cyclic AMP agonists that promote the movement of lysosomes toward the cell periphery and facilitate Ca^{2+}-regulated lysosome exocytosis enhance *T. cruzi* invasion of fibroblasts (Rodríguez *et al.*, 1999). Infective *T. cruzi* trypomastigotes activate adenyl cyclase in mammalian cells to generate increased levels of cAMP (Rodríguez *et al.*, 1999) (Fig. 2, pathway 6) and pharmacologic blockers of this pathway inhibit parasite entry. Notably, the residual capacity for host cell invasion by the *T. cruzi opb–/–* mutants could be restored to near wild-type levels by stimulating cells with isopreterenol or 8-bromo-cAMP (Caler *et al.,* 2000), suggesting that, while both Ca^{2+}- and cAMP-signalling pathways participate in lysosome-mediated entry, these pathways are triggered by distinct parasite-dependent mechanisms (Caler *et al.,* 2000). The parasite molecules responsible for activating host cell adenyl cyclase remain to be identified.

Phosphatidylinositol (PI)-3 kinases

Phosphatidylinositol-3 kinases are activated by a wide variety of extracellular stimuli and regulate distinct cellular processes through the generation of specific lipid products (Toker and Cantley, 1997). Modulation of host cell PI-3 kinase-dependent signalling by microbial pathogens is an important strategy for establishment of infection. Actin-dependent entry of non-professional phagocytic cells by bacterial pathogens such as *Yersinia* (Schulte *et al.*, 1998) and *Listeria monocytogenes* (Ireton *et al.*, 1996) is regulated by host cell PI-3 kinases, while subversion of PI-3 kinase signalling allows the extracellular pathogen enteropathogenic *E. coli* to avoid cellular uptake by phagocytosis (Celli *et al.,* 2001). It was recently demonstrated that host cell PI-3 kinase and Akt (a serine/threonine kinase) are important regulators of *T. cruzi* invasion of non-professional phagocytes (Wilkowsky *et al.,* 2001) as well as macrophages (Todorov *et al.,* 2000). Because PI-3 kinases regulate actin-dependent processes (Rodriguez-Viciana *et al.*, 1997; Conejo and Lorenzo, 2001) as well as regulated exocytosis (Yang *et al.*, 1996), this signalling pathway may function as a modulator of lysosome-mediated invasion or potentially regulate an actin-associated entry process (Procopio *et al.*, 1999).

In addition to facilitating invasion, activation of host cell PI-3 kinase/Akt signalling was recently shown to stimulate pro-survival pathways in *T. cruzi*-infected neuronal cells (Chuenkova *et al.,* 2001). This effect was shown to be mediated by the abundant trypomastigote surface glycoprotein, *trans*-sialidase (TS) (Chuenkova *et al.*, 2001). Together with the related gp85 proteins, TS family members form a population of heterogeneous glycosylphosphatidylinositol (GPI)-anchored trypomastigote surface glycoproteins that play an important role in initial host cell recognition and attachment (described in more detail below). TS activity has also been implicated in facilitating disruption of the *T. cruzi* vacuole by liberating sialic acids from the heavily glycosylated lysosome-associated membrane proteins (Hall *et al.*, 1992). Collectively, this diverse group of related *T. cruzi* surface glycoproteins mediate intimate association of infective trypomastigotes with a wide variety of mammalian cell types, activate PI-3 kinase-dependent signalling, to promote parasite invasion and host cell survival.

Tyrosine phosphorylation

Protein tyrosine phosphorylation is an important feature in the regulation of a variety of different eukaryotic signalling pathways including PI-3-kinase activation (Porter and Vaillancourt, 1998; Cary *et al.*, 1999; Storz and Toker, 2002). In professional phagocytes, *T. cruzi* stimulates tyrosine phosphorylation of a number of proteins (Ruta *et al.*, 1996) and macrophage entry is inhibited by the tyrosine kinase inhibitor genestein (Vieira *et al.*, 1994). In contrast, dephosphorylation of tyrosine has been correlated with infectivity of rat myoblasts, where inhibitors of tyrosine phosphate hydrolysis significantly reduce efficiency of host cell invasion (Zhong *et al.*, 1998). In addition, genestein pretreatment of NRK fibroblasts failed to inhibit parasite entry (Rodríguez *et al.*, 1995). These data suggest differential roles for protein tyrosine kinase and phosphatase activities in promoting *T. cruzi* entry of professional and non-professional phagocytic cells respectively. In this regard, it is intriguing to note that infective trypomastigotes as well as intracellular amastigotes express an ecto-tyrosine phosphatase activity (Furuya *et al.*, 1998). Whereas the role of this enzymatic activity during trypomastigote invasion has not been elucidated, access to tyrosine phosphorylated substrates by intracellular amastigotes in the host cell cytoplasm may profoundly influence the phosphorylation state of many cellular proteins during the course of infection.

Inhibitory effects of NF-κB activation on *T. cruzi* invasion

Early signalling events elicited in the host cell by *T. cruzi* are predicted to evoke changes in host cell gene expression. A frequent consequence of mammalian cell invasion by intracellular pathogens is the activation of host cell NF-κB signalling pathways (Philpott *et al.*, 2000; Heussler *et al.*, 2001; Wahl *et al.*, 2001). The NF-κB family of transcription factors regulate the expression of a large number of genes that participate in the management of host inflammatory and immune responses (Ghosh *et al.*,

1998; Karin and Delhase, 2000). *Trypanosoma cruzi*-mediated NF-κB activation and induction of pro-inflammatory cytokine synthesis has been extensively studied in macrophages (reviewed by Ropert *et al.,* 2002), where signalling through Toll-like receptor 2 by GPI-anchored mucin-like glycoproteins derived from infective trypomastigotes initiates the response (Campos *et al.,* 2001). In non-professional phagocytic cells, an inverse correlation between transcription factor NF-κB activation in mammalian cells and susceptibility to *T. cruzi* infection has been reported (Hall *et al.,* 2000). Parasite-dependent NF-κB activation was not observed in myocytes for which *T. cruzi* exhibits tropism *in vitro* and *in vivo*, whereas nuclear translocation and DNA binding of the p65 subunit of NF-κB and expression of an NF-κB-dependent reporter construct was observed in epithelial cells, endothelial cells, and fibroblasts (Hall *et al.,* 2000). In this study, inhibition of NF-κB signalling was shown to significantly enhance *T. cruzi* invasion of epithelial cells suggesting that differential activation of this pathway in different cell types may determine tissue tropism in the host, a process that is poorly understood. The universality of these findings is challenged by studies in which NF-κB activation in isolated cardiomyocytes was implicated by the induction of pro-inflammatory cytokine gene expression following *T. cruzi* infection (Machado *et al.,* 2000). Furthermore, a recent DNA microarray analysis that examined global transcriptional responses in primary fibroblasts to *T. cruzi* revealed no induction of pro-inflammatory cytokines at 24 h post infection (Vaena de Avalos *et al.,* 2002), suggesting that NF-κB was not activated by the parasite in this cell type. Although the role of NF-κB was not directly tested in these studies (Machado *et al.,* 2000; Vaena de Avalos *et al.,* 2002), the results suggest that cell type-specific activation of this transcription factor may not be a reliable predictor of tissue tropism. Nevertheless, differential activation of host cell NF-κB in response to *T. cruzi* may profoundly influence the local cytokine environment in the host during infection.

Host cell receptors

Receptors for the T. cruzi trans-*sialidase/gp85 family*

Initiation of communication between *T. cruzi* trypomastigotes and mammalian cells requires contact of parasite ligands (soluble or surface membrane bound) with host cell receptors. Although the role for host cell surface sialic acid containing molecules and heparan sulphate proteoglycans has long been recognized (Ming *et al.,* 1993; Schenkman *et al.,* 1993; Herrera *et al.,* 1994), definitive receptors that mediate *T. cruzi* attachment and/or signalling have been elusive. Recent evidence suggests that host cell recognition is mediated by diverse families of parasite surface glycoproteins which permit interaction with distinct host cell receptors and extracellular matrix molecules (Alves *et al.,* 1986; Abuin *et al.,* 1989; Ming *et al.,* 1993; Giordano *et al.,* 1994). The TS/gp85 family contains fibronectin-like sequences (Pereira *et al.,* 1991) and a laminin-binding domain (Giordano *et al.,* 1994) implicating cellular integrins in the invasion process. In addition, a conserved sequence found in the C-terminal part of the protein that binds specifically to cytokeratin 18 has recently been identified (Magdesian *et al.,* 2001). Integrins that function as cellular laminin or fibronectin receptors activate PI-3 kinase signalling pathways (Shaw, 2001) and promote cell survival through activation of *bcl-2* transcription. Because a similar activity has already been ascribed to *T. cruzi trans*-sialidase (Chuenkova *et al.,* 2001), it is possible that these signalling events are transduced through integrins. As a substrate for protein kinase C (PKCδ) (Omary *et al.,* 1992; Busso *et al.,* 1994) cytokeratin 18 could also function to transduce signals from the cell surface following binding of gp85. Neither of these hypotheses has been directly tested.

Role of host TGF-β receptors

Signal transduction through TGF-β receptors facilitates *T. cruzi* entry of epithelial cells (Ming *et al.,* 1995; Hall and Pereira, 2000). Infective trypomastigotes and parasite-conditioned medium can induce expression of a TGF-β-responsive plasminogen activator inhibitor-1 reporter gene construct (Fig. 2, pathway 1) whereas cell lines deficient in receptor types I or II (RI, RII) were refractory to *T. cruzi* infection (Ming *et al.,* 1995). These observations led to the proposal that infective stages of *T. cruzi* secrete a TGF-β-like molecule or a factor capable of activating latent host TGF-β (Ming *et al.,* 1995). However such a molecule has not been identified.

In support of a role for functional TGF-β receptors for *T. cruzi* infection, exogenous addition of TGF-β to cells or overexpression of constitutively active Smad2 dramatically enhanced trypomastigote invasion of epithelial cells (Hall and Pereira, 2000). Cellular Smad proteins are the only TGF-β receptor kinase substrates with a demonstrated ability to transduce signals from the activated receptors (Massague, 1998). Interestingly, expression of inhibitory Smads such as Smad 7 or a dominant-interfering Smad2 mutant failed to inhibit basal level *T. cruzi* invasion, although these reagents effectively abolished TGF-β-mediated enhancement of parasite entry (Hall and Pereira, 2000). Thus, while kinase-active TGF-β R1 appears to be required for parasite invasion of epithelial cells, downstream signalling events do not appear to be Smad-dependent. Whereas it is possible that some of the other receptor-Smads not examined in this study (Hall and Pereira, 2000) may be involved in *T. cruzi* entry,

the authors concluded that functional TGF-β receptors play a limited role during establishment of infection by mediating the invasion step, but their signalling function is not required for parasite attachment or for intracellular growth and survival (Hall and Pereira, 2000). As TGF-β exerts an important immunomodulatory role in acute and chronic Chagas' disease where relatively high levels of this cytokine have been observed in *T. cruzi*-infected hearts (Zhang and Tarleton, 1996), the ability of TGF-β to positively regulate parasite invasion may have important consequences for disease pathogenesis. These aspects require further investigation.

Receptor for the oligopeptidase B-dependent Ca²⁺-agonist

The participation of a $G_{\alpha i}$ or $G_{\alpha o}$ heterotrimeric G-protein coupled receptor for the putative Opb-generated Ca^{2+}-agonist has been postulated due to the sensitivity of this signalling pathway to pertussis toxin (PTx) (Tardieux *et al.*, 1994) and to mild trypsinization of the host cell surface (Leite *et al.*, 1998). A wide variety of mammalian cell types are sensitive to the soluble Opb-dependent Ca^{2+}-signalling activity (Burleigh and Andrews, 1995b), suggesting that receptors for the putative Opb-dependent agonist are commonly expressed. Heterologous expression of rat fibroblast mRNA in *Xenopus* oocytes, which do not display an endogenous Ca^{2+}-response to *T. cruzi* extracts, was shown to confer sensitivity of the oocytes to the trypomastigote peptidase-dependent Ca^{2+}-signalling activity (Leite *et al.*, 1998). The characteristics of the oocyte response were similar to that demonstrated for mammalian cells, i.e. sensitive to inhibitors of Opb as well as PTx or thapsigargin pretreatment (Leite *et al.*, 1998), demonstrating the potential of this heterologous expression system for cloning of the mammalian receptor for the Opb-generated Ca^{2+}-agonist.

Aside from the roles of host cell bradykinin (B_2K) receptors in transducing Ca^{2+}-signals from cruzipain-processed kinins (Scharfstein *et al.,* 2000), and the involvement of TGF-β receptors in epithelial cell invasion (Ming *et al.*, 1995), no definitive host cell receptors for *T. cruzi*-induced signalling have been identified. With the expression of such diverse surface glycoprotein ligands, secreted proteases and putative signalling agonists, the range of potential host cell receptors is predicted to be very broad and may confound efforts to definitively identify functional receptors due to the potential for redundant signalling mechanisms.

Concluding remarks

Major concepts emerging from recent studies on *T. cruzi* invasion highlight the enormous complexity inherent in the mechanisms required for establishment of infection by this pathogen. Infective trypomastigotes exploit important cellular processes including wound-repair mechanisms and anti-apoptotic functions for invasion and intracellular survival. Active manipulation of multiple mammalian cell signalling pathways is mediated by direct parasite–host interactions and soluble secreted factors. Expression of abundant heterogenous glycoproteins on the surface of *T. cruzi* trypomastigotes with affinities for mammalian extracellular matrix proteins ensures access to a broad spectrum of mammalian cell types during the acute stages of infection. Despite recent progress in the field, it is not yet clear how *T. cruzi*-induced signalling pathways are integrated to co-ordinate invasion, support intracellular replication or influence tissue tropism. Studies outlined above provide the foundation for future mechanistic studies that are essential to our understanding of the molecular basis for *T. cruzi* infection and pathogenesis of Chagas' disease.

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