Brief Definitive Report

Role in Host Cell Invasion of *Trypanosoma cruzi*-induced Cytosolic-free Ca^{2+} Transients

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Summary

Trypanosoma cruzi enters cells by a unique mechanism, distinct from phagocytosis. Invasion is facilitated by disruption of host cell actin microfilaments, and involves recruitment and fusion of host lysosomes at the site of parasite entry. These findings implied the existence of transmembrane signaling mechanisms triggered by the parasites in the host cells before invasion. Here we show that infective trypomastigotes or their isolated membranes, but not the noninfective epimastigotes, induce repetitive cytosolic-free Ca^{2+} transients in individual normal rat kidney fibroblasts, in a pertussis toxin–sensitive manner. Parasite entry is inhibited by buffering or depleting host cell cytosolic-free Ca^{2+}, or by pretreatment with Ca^{2+} channel blockers or pertussis toxin. In contrast, invasion is enhanced by brief exposure of the host cells to cytochalasin D. These results indicate that a trypomastigote membrane factor triggers cytosolic-free Ca^{2+} transients in host cells through a G-protein–coupled pathway. This signaling event may promote invasion through modulation of the host cell actin cytoskeleton.

The protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease, can invade a variety of vertebrate cells. Invasion occurs without apparent pseudopod formation and host cell actin polymerization, raising questions regarding the origin of the membrane required to form the parasite-containing vacuoles. Recent observations showed that host cell lysosomes cluster underneath the parasite's attachment site at the plasma membrane, and that lysosomal fusion occurs at very early stages of the invasion process (1, 2). Fusion of lysosomes is required for *T. cruzi* entry, suggesting that lysosomes may serve as a source of membrane to form the intracellular vacuole. Since trypanosome entry is significantly enhanced by brief exposure of the host cells to cytochalasin D, removal of the cortical cytoskeleton barrier may be an early step in the invasion process, facilitating lysosome access and fusion to the plasma membrane (1–4).

The mobilization of host cell lysosomes observed during *T. cruzi* invasion implies that a transmembrane signal must be generated upon binding of the parasites to host cells. Since Ca^{2+} regulates cytoskeletal rearrangements (5–7) and exocytosis (8–10) in a number of cell types, we examined the role of Ca^{2+} signaling in the interaction of *T. cruzi* trypomastigotes with rat fibroblasts. We show that rapid, repetitive cytosolic Ca^{2+} elevations occur in the host cells as soon as they are exposed to trypomastigotes, and that the same pattern of signaling is elicited by isolated parasite membranes. The *T. cruzi*-induced Ca^{2+} signaling may play a role in priming the host cells for invasion, since it is detected before parasite internalization, a process known to require 8–10 min to be completed (11).

Materials and Methods

Materials. Trypsin, soybean trypsin inhibitor (SBTI), pertussis toxin (PTx), cholera toxin (CTx), epidermal growth factor (EGF), A23187, cytochalasin D, verapamil, $NiCl_2$, Hepes and EGTA were obtained from Sigma Chemical Co. (St. Louis, MO), and MAPTA-AM and fluo-3 were obtained from Molecular Probes, Inc. (Eugene, OR).

Cells and Parasites. Normal rat kidney (NRK) cells were grown in 10 mM Hepes-buffered DMEM containing 5% FBS, at 37°C in a 5% CO_2 atmosphere. Trypomastigotes from the *T. cruzi* Y strain were obtained from the supernatant of infected LLC-MK_2 supernatants (1, 12). Epimastigotes from the Y strain were cultured in liver infusion tryptose medium containing 10% FBS at 28°C (13). *T. brucei* procyclics ITat 1.1 were grown in Cunningham's medium (14) supplemented with 10% FBS and 25 mM Hepes, at 28°C. For some experiments, parasites were killed by a 3-min incubation at 56°C. Parasites were washed in Hepes-buffered Ringer's solution (15) and resuspended in the same solution at 10^8/ml for time lapse confocal microscopy or invasion experiments.

Time Lapse Confocal Microscopy. NRK rat fibroblasts seeded on glass coverslips at a density of $2.5 \times 10^6/cm^2$ were loaded with 5 μM fluo-3/AM for 30 min at 37°C (16), transferred to a heated chamber on the stage of a Zeiss Axiovert microscope, perfused at 37°C with Hepes-buffered Ringer's solution, and observed using a confocal imaging system (MRC-600; Bio-Rad Laboratories, Cambridge, MA), taking optical sections of approximately 2 μm in thick-

ness (17). Parasites were introduced into the chamber in Ringer's solution at a density of 10^8/ml. Images were recorded at a rate of one frame per second on an optical memory disk (model TQ3031F; Panasonic, Seacaucus, NJ) and analyzed subsequently using an image processor (Series 151; Itex, Woburn, MA) (17).

Cell Treatments. NRK cells were pretreated with 0.4 μg/ml PIx for 4 h at 37°C, before exposure to parasites or isolated membranes. Parasite membranes were prepared as described previously (18) and resuspended in Ringer's buffer at 10^8 parasite equivalents/ml. Isolated membranes were treated with 150 μg/ml trypsin for 30 min

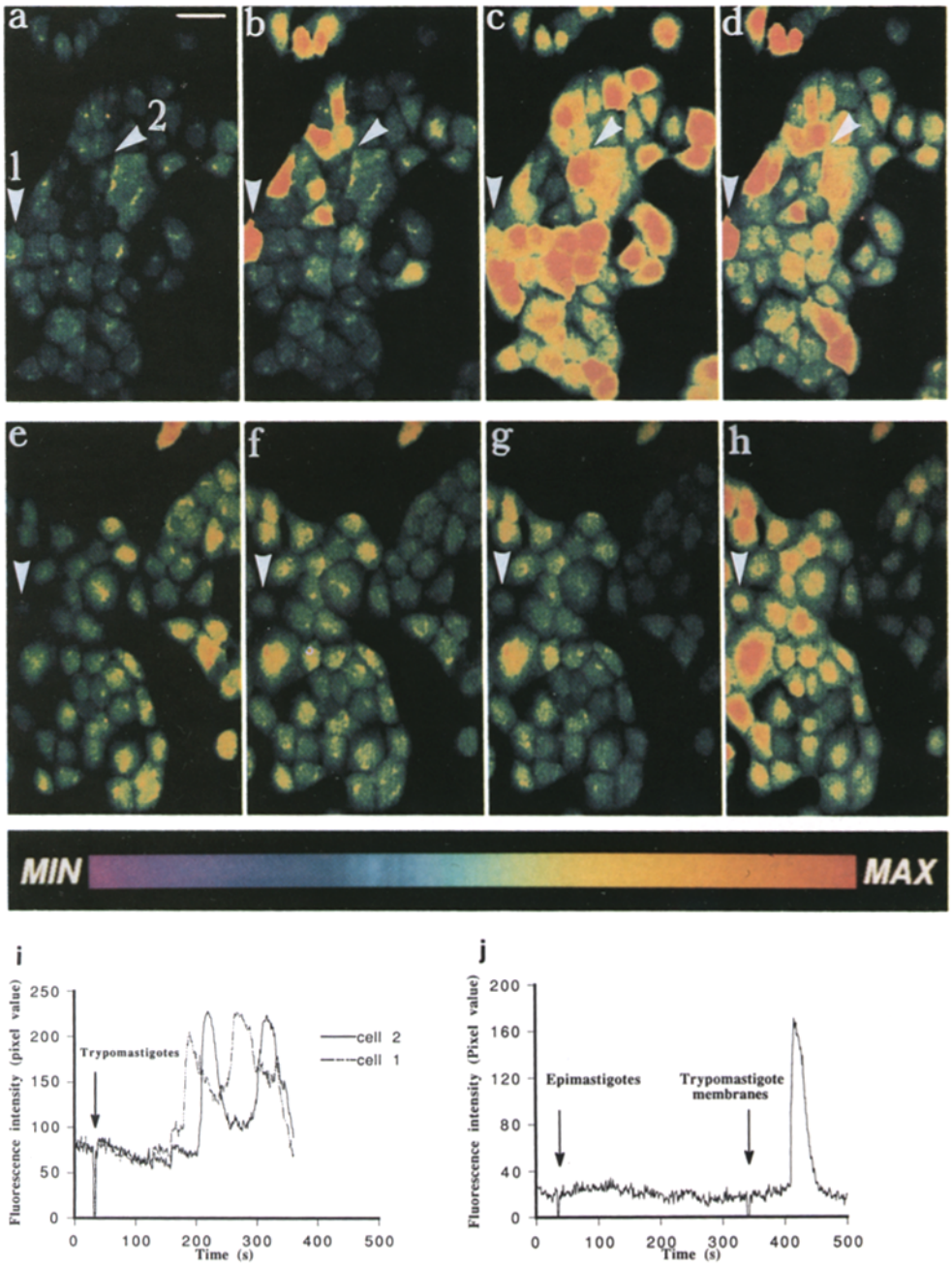
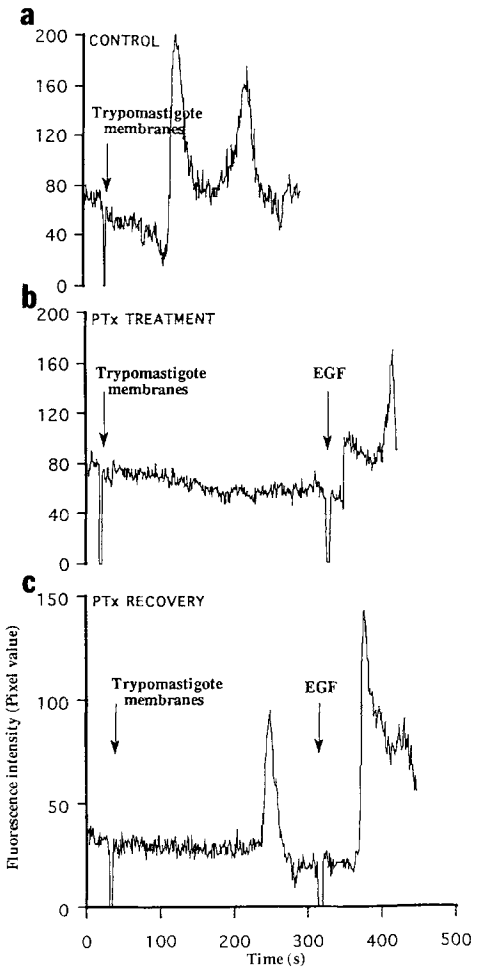


Figure 1. Subcellular changes in cytosolic Ca^{2+} in NRK cells exposed to distinct life cycle stages of *T. cruzi*. (*a–d*) Serial confocal microscopic images of NRK fibroblasts loaded with the Ca^{2+}-sensitive dye fluo-3 and exposed to live, infective trypomastigotes. (*a*) Baseline image before exposure to *T. cruzi*. (*b–d*) Same field 200, 250, and 300 s after exposure to trypomastigotes. (*e–h*) Serial images of a different group of fibroblasts after exposure to live, noninfective epimastigotes. (*e–g*) Images before, 150, and 300 s after exposure to epimastigotes. (*h*) Same field after subsequent exposure to trypomastigote membranes. Fluorescence intensity in *a–h* are pseudocolored according to the color scale located below. Large increases in fluorescence intensity correspond to increasing $[Ca^{2+}]_i$, but subtle fluctuations in fluorescence intensity also occur because fluo-3 cannot be ratio-imaged (37). Scale bar (*top left*) is 25 μm. (*i*) Graphical representation of fluorescence intensity over time in rectangular regions within the two cells indicated in *a–d* (*arrowheads*). The increase in $[Ca^{2-}]_i$ occur in each cell ~100 s apart, in a repetitive and asynchronous fashion. (*j*) Graphical representation of fluorescence intensity over time in a rectangular region within the cell indicated in *e–g* (*arrowhead*). An increase in $[Ca^{2+}]_i$ occurred only after the cell was exposed to trypomastigote membranes.



at 37°C, followed by 150 μg/ml SBTI. Control membranes were treated only with SBTI. EGF was added in each experiment after about 4 min of image recording, at a final concentration of 5 nM.

Invasion Assays. The number of intracellular parasites was determined after a 20-min infection period, as detailed previously (1). Host cell pretreatments were performed at 37°C, as follows: 10 min with Ca^{2+}-free medium, 1 h with 500 μM MAPTA-AM, 5 min with 0.5 μM A23187, 15 min with 5 mM $NiCl_2$, 30 min with 100 μM verapamil, or 4 h with 0.4 μg/ml PTx or CTx. All drugs were removed before exposure of the cells to parasites in invasion assays. Where not stated, the free Ca^{2+} concentration in the medium was 1 mM. The Ca^{2+}-free medium contained 5 mM EGTA. Ca^{2+} conditions in the medium were kept constant during host cell pretreatment and infection.

Results and Discussion

The effects of two distinct life cycle stages of *T. cruzi* on Ca^{2+} signaling in NRK fibroblasts were examined using confocal video microscopy. Shortly after being introduced to the chamber, the infective trypomastigote forms induced repetitive increases in cytosolic-free calcium ($[Ca^{2+}]_i$) in in-

Figure 2. Effect of pertussis toxin on cytosolic Ca^{2+} signals induced by trypomastigote membranes. (*a–c*) Graphical representation of fluorescence intensity over time in single NRK cells after: (*a*) no pretreatment and exposure to isolated trypomastigote membranes. Note repetitive increases in $[Ca^{2+}]_i$ occurring 100 s apart. (*b*) Pretreatment with PTx followed by exposure to trypomastigote membranes. No increase in $[Ca^{2+}]_i$ is detected. Subsequent addition of EGF elicits a Ca^{2+} signal. (*c*) Pretreatment with PTx followed by a recovery period of 1 h, then exposure to trypomastigote membranes. Unlike cells with no recovery period, a Ca^{2+} signal is detected in these cells. Subsequent addition of EGF again induces a Ca^{2+} signal.

Table 1. *Ca^{2+} Signaling Induced in NRK Cells by Trypanosomes*

Experiments	1	2	3	4	5	6
T. cruzi trypomastigote	20/85 (23%)*	15/50 (30%)	20/60 (33%)	6/34 (17.6%)	7/35 (20%)	12/38 (32%)
T. cruzi epimastigote	0/45 (0%)	0/60 (0%)	0/52 (0%)			
T. brucei procyclic	0/55 (0%)	0/45 (0%)				
Trypomastigote membranes	40/46 (86%)	20/45 (44%)	11/22 (50%)	35/65 (54%)	19/45 (42%)	
Epimastigote membranes	0/45 (0%)					
Trypsin-treated trypomastigote membranes	1/55 (2%)	0/43 (0%)				
EGF	45/52 (86%)	52/55 (94%)	48/48 (100%)			
PTx treated/ trypomastigote membranes	0/55 (0%)	0/45 (0%)				
PTx treated + recovery/ trypomastigote membranes	3/45 (7%)‡	6/55 (11%)‡	13/40 (32%)§			

Recorded images obtained as described in Materials and Methods were observed frame by frame and the number of cells showing at least one Ca^{2+} spike during a period of 400 s was determined.

* Values represent (number of cells with a Ca^{2+} spike)/(total number of cells observed).

‡ 30 min after toxin removal.

§ 60 min after toxin removal.

dividual fibroblasts (Fig. 1, *a–d*, and *i*). $[Ca^{2+}]_i$ increases occurred within 200 s of exposure but were asynchronous, suggesting they were triggered by parasite attachment to individual cells. Trypomastigotes are 10–20 μm long, and at the density used (10^8/ml), several could be visualized attached to some cells of the population. Invasion is a slower process, requiring at least 10 min of trypomastigote–host cell interaction (11). $[Ca^{2+}]_i$ responses were also elicited by heat-killed (3 min at 55°C) trypomastigotes, or by membrane fractions isolated from trypomastigotes (Figs. 1, *h* and *j* and 2 *a*). Treatment of the trypomastigote membranes with trypsin abolished their capacity to trigger $[Ca^{2+}]_i$ signals in NRK cells (Table 1). Epimastigotes, the *T. cruzi* life cycle stages which are not capable of invading vertebrate cells (13), did not induce $[Ca^{2+}]_i$ signaling (Fig. 1, *e–g*, and *j*), and neither did their isolated membranes (Table 1). Negative results were also obtained with procyclics of *T. brucei*, the exclusively extracellular African trypanosome (Table 1).

The induction of $[Ca^{2+}]_i$ transients exclusively by the infective trypomastigote forms suggested that Ca^{2+} signaling was necessary for invasion. Accordingly, the cells were treated in several ways to inhibit the $[Ca^{2+}]_i$ signals, and then exposed to parasites in invasion assays. Trypomastigote entry was significantly impaired by preloading the host cells with the membrane-permeant Ca^{2+} chelator MAPTA-AM, which acts as a buffer, clamping the $[Ca^{2+}]_i$ at resting levels (19) (Fig. 3). Depletion of host cell $[Ca^{2+}]_i$ before exposure to the parasites, by exposure of the fibroblasts to the Ca^{2+} ionophore A23187 (20) or to the intracellular Ca^{2+} chelator MAPTA-AM (21) in Ca^{2+}-free medium, further inhibited trypanosome entry (Fig. 3). The decrease in infection rate induced by A23187 in the presence of Ca^{2+} was of marginal significance ($p = 0.02$). Since addition of the ionophore should have equilibrated the intracellular and extracellular Ca^{2+} concentrations, this suggests that, like parasite-induced transient Ca^{2+} increases, a sustained ionophore-induced Ca^{2+} increase is also permissive for *T. cruzi* invasion. Under each of the inhibitory conditions, however, parasite entry was still increased two- to threefold by pretreatment of the host cells for 5 min with cytochalasin D (legend to Fig. 3). Removal of Ca^{2+} from the medium before and during infection also inhibited *T. cruzi* invasion (Fig. 3). This is not likely to reflect metabolic changes in the parasites, since the concentration of free Ca^{2+} in the cytoplasm of *T. cruzi* trypomastigotes is maintained at 10–20 nM, regardless of the presence or absence of external Ca^{2+} (22). Pretreatment of the host cells with the Ca^{2+} channel blockers $NiCl_2$ (23) or verapamil (24) also markedly reduced *T. cruzi* invasion (Fig. 3). NRK cells treated with $NiCl_2$ did not respond with Ca^{2+} signals when exposed to trypomastigote membranes (data not shown). These findings are consistent with a requirement for $[Ca^{2+}]_i$ increases in the host cells for *T. cruzi* entry, and suggest that Ca^{2+} influx is a necessary component of the parasite-induced signaling process. It is interesting to note that verapamil treatment has been reported to dramatically improve survival and myocardial disease in *T. cruzi*–infected mice (25).

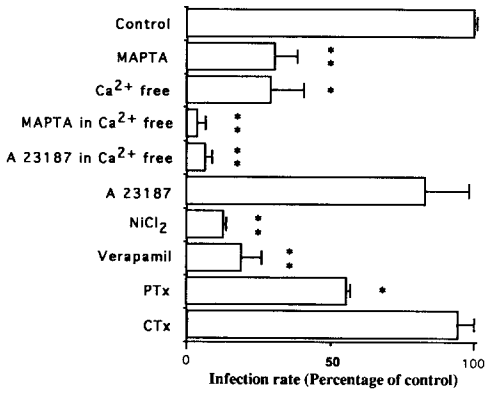


Figure 3. Effect of inhibitors of Ca^{2+} signaling on the invasion of NRK cells by *T. cruzi* trypomastigotes. Effect of buffering (*MAPTA*) or depleting (MAPTA/Ca^{2+}-free and A23187/Ca^{2+}-free) host cell $[Ca^{2+}]_i$, of blocking plasma membrane Ca^{2+} channels ($NiCl_2$ and verapamil), or of pretreatment with PTx or CTx before infection. Each condition, except A23187 in the presence of Ca^{2+} and CTx, significantly inhibits invasion. (*) $p < 0.005$; (**) $p < 0.0001$. Values shown are the mean ± SD of triplicate samples from representative experiments. In matched experiments, cytochalasin D (2 μM) was added during the last 5 min of the host cell pretreatment. The ratios (number of intracellular parasites with cytochalasin pretreatment)/(number of intracellular parasites without cytochalasin pretreatment) were (*control*) 2.0; (*MAPTA*) 2.7; (*Ca^{2+} free*) 2.0; (*MAPTA in Ca^{2+} free*) 3.2; and (*A23187 in Ca^{2+} free*) 3.2; Ptx: 3.3.

It is noteworthy that the protocols used by us to buffer or deplete $[Ca^{2+}]_i$ do not interfere with particle ingestion by phagocytosis, in macrophages (26) or neutrophils (27, 28). In contrast, there is evidence that $[Ca^{2+}]_i$ transients are required for phagosome-lysosome fusion (28). Our finding that $[Ca^{2+}]_i$ transients are involved in *T. cruzi* entry into fibroblasts is therefore consistent with invasion being mediated by lysosomal fusion (1).

A role for heterotrimeric G proteins in signal transduction is well established (29). Treatment of intact cells with PTx results in ADP-ribosylation of members of a restricted class of G protein α-subunits, uncoupling them from their receptors and thus blocking signal transduction (30, 31). The PTx-sensitive G-protein subtypes $G\alpha_i$ and $G\alpha_o$ are involved in the regulation of Ca^{2+} influx channels, and in the activation of phospholipase C, which generates IP_3-mediated Ca^{2+} release from internal stores (29, 31, 32). Incubation of NRK fibroblasts with PTx for 4 h inhibited their $[Ca^{2+}]_i$ response to *T. cruzi* (Fig. 2 *b*). However, PTx-treated cells still responded normally to EGF, which induces $[Ca^{2+}]_i$ transients by interaction with a receptor tyrosine kinase, in a pathway independent of trimeric G proteins (33) (Fig. 2 *b*). The inhibitory effect of PTx on the $[Ca^{2+}]_i$ signal triggered by trypomastigote membranes was partially reversed after recovery periods of 30 min to 1 h (Table 1, Fig. 2 *c*). Taken together, these findings indicate that a PTx-sensitive host cell G protein is involved in the regulation of the Ca^{2+} signals elicited by *T. cruzi*.

Invasion of NRK fibroblasts by *T. cruzi* trypomastigotes was inhibited when the cells were pretreated with PTx (Fig. 3). The partial inhibition is probably due to recovery of the fibroblast population from PTx, since the toxin was removed before the 20-min infection period (see Table 1). CTx, which

ADP-ribosylates the α-subunit of Gs but not of Gi trimeric G proteins (34), affected neither invasion (Fig. 3) nor the $[Ca^{2+}]_i$ transients induced by trypomastigote membranes (data not shown). Thus, *T. cruzi* invasion depends on Ca^{2+} signals elicited by interaction between the parasite membrane and a host cell surface component coupled to a pathway including a PTx-sensitive G protein. It is interesting to note that exposure of the PTx-treated fibroblasts to cytochalasin D for 5 min enhanced invasion to levels similar to those observed in control cells (legend to Fig. 3), suggesting that the action of the toxin can in large part be bypassed by disassembly of the actin cytoskeleton.

Ca^{2+} signaling also appears to be important for cell entry by *Salmonella typhimurium*. Increases in $[Ca^{2+}]_i$ were detected in populations of Henle-407 cells exposed to this bacterium, and Ca^{2+} channel blockers had an inhibitory effect in invasion (35). Although observations at the single cell level still have to be performed to confirm these findings, it is possible that $[Ca^{2+}]_i$ transients are involved in regulating the extensive cytoskeletal rearrangements known to accompany *Salmonella* invasion. The *Salmonella* mechanism for cell invasion is clearly distinct from the one used by *T. cruzi*, since it is dependent on host cell membrane ruffling and inhibited by cytochalasins (36). Further characterization of the $[Ca^{2+}]_i$ fluxes occurring in these two systems is needed to clarify the possible involvement of distinct signaling pathways.

In view of our previous findings (1) cytosolic-free Ca^{2+} transients may be required for at least two steps of the *T. cruzi* invasion process: (*a*) local rearrangement of the cortical actin cytoskeleton allowing lysosome access to the plasma membrane, and (*b*) lysosome fusion at the site of trypanosome entry. Characterization of the trypomastigote ligand and the host cell receptor mediating the $[Ca^{2+}]_i$ response may reveal a specific signal transduction pathway that couples cytoskeletal organization and membrane traffic.

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References

1. Tardieux, I., P. Webster, J. Ravesloot, W. Boron, J.A. Lunn, J.E. Heuser, and N.W. Andrews. 1992. Lysosome recruitment and fusion are early events required for trypanosome invasion of mammalian cells. *Cell.* 71:1117.
2. Hall, B.F., P. Webster, A.K. Ma, K.A. Joiner, and N.W. Andrews. 1992. Desialylation of lysosomal membrane glycoproteins by *Trypanosoma cruzi*: a role for the surface neuraminidase in facilitating parasite entry into the host cell cytoplasm. *J. Exp. Med.* 176:313.
3. Schenkman, S., N.W. Andrews, V. Nussenzweig, and E.S. Robbins. 1988. Trypanosoma cruzi invade a mammalian epithelial cell in a polarized manner. *Cell.* 55:157.
4. Schenkman, S., E.S. Robbins, and V. Nussenzweig. 1991. Attachment of *Trypanosoma cruzi* to mammalian cells requires parasite energy, and invasion can be independent of the target cell cytoskeleton. *Infect. Immun.* 59:645.
5. Bretscher, A. 1991. Microfilament structure and function in the cortical cytoskeleton. *Annu. Rev. Cell Biol.* 7:337.
6. Aderem, A. 1992. Signal transduction and the actin cytoskeleton: the roles of MARKS and profilin. *TIBS (Trends Biochem. Sci.).* 17:438.
7. Stossel, T.P. 1993. On the crawling of animal cells. *Science (Wash. DC).* 260:1086.
8. Trifaró, J.M., A. Rodriguez del Castillo, and M.L. Vitale. 1992. Dynamic changes in chromaffin cell cytoskeleton as prelude to exocytosis. *Molecular Neurobiology.* 6:339.
9. Llinas, R., M. Sugimori, and R.B. Silver. 1992. Microdomains of high calcium concentration in a pre-synaptic terminal. *Science (Wash. DC).* 256:677.
10. Thomas, P., J.G. Wong, and W. Almers. 1993. Millisecond studies of secretion in single rat pituitary cells stimulated by flash photolysis of caged Ca^{2+}. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:303.
11. Dvorak, J.A., and T.P. Hyde. 1973. *Trypanosoma cruzi*: interaction with vertebrate cells in vitro. I. Individual interactions at the cellular and subcellular levels. *Exp. Parasitol.* 34:268.
12. Andrews, N.W., K.S. Hong, E.S. Robbins, and V. Nussenzweig. 1987. Stage-specific surface antigens expressed during the morphogenesis of *Trypanosoma cruzi*. *Exp. Parasitol.* 64:474.
13. Nogueira, N., and Z. Cohn. 1976. *Trypanosoma cruzi*: mechanism of entry and intracellular fate in mammalian cells. *J. Exp. Med.* 143:1402.
14. Cunningham, I. 1977. New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. *J. Protozol.* 24:325.
15. Heuser, J. 1989. Changes in lysosome shape and distribution correlated with changes in cytoplasmic pH. *J. Cell Biol.* 108:855.
16. Nathanson, M.H., and A.D. Burgstahler. 1992. Subcellular distribution of cytosolic Ca^{2+} in isolated rat hepatocyte couplets: evaluation using confocal microscopy. *Cell Calcium.* 13:89.

17. Nathanson, M.H., M.S. Moyer, A.D. Burgstahler, A.-M. O'Carroll, M.J. Brownstein, and S.J. Lolait. 1992. Mechanisms of subcellular cytosolic Ca^{2+} signalling evoked by stimulation of the vasopressin V_{1a} receptor. *J. Biol. Chem.* 267:23282.
18. Ortega-Barria, E., and M.E.A. Pereira. 1991. A novel *T. cruzi* heparin-binding protein promotes fibroblast adhesion and penetration of engineered bacteria and trypanosomes into mammalian cells. *Cell.* 67:411.
19. Huang, A.J., J.E. Manning, T.M. Bandak, M.C. Ratau, K.R. Hanser, and S.C. Silverstein. 1993. Endothelial cell cytosolic free calcium. *J. Cell Biol.* 120:1371.
20. Lew, V.L., and J. Garcia-Sancho. 1985. Use of the ionophore A23187 to measure and control cytoplasmic Ca^{2+} levels in intact red cells. *Cell Calcium.* 6:15.
21. Marks, P.W., B. Hendey, and F.R. Maxfield. 1991. Attachment to fibronectin or vitronectin makes human neutrophil migration sensitive to alterations in cytosolic free calcium concentration. *J. Cell Biol.* 112:149.
22. Docampo, R., S.N.J. Moreno, and A. Vercesi. 1993. Effect of thapsigargin on calcium homeostasis in *Trypanosoma cruzi* trypomastigotes and epimastigotes. *Mol. Biochem. Parasitol.* 59: 305.
23. Hagiwara, S., and L. Byerly. 1981. Calcium channels. *Annu. Rev. Neurosci.* 4:69.
24. Reuter, H. 1983. Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature (Lond.).* 301:569.
25. Morris, S.A., L.M. Weiss, S. Factor, J.P. Bilezikian, H. Tanowitz, and M. Wittner. Verapamil ameliorates clinical, pathologic and biochemical manifestations of experimental chagasic cardiomyopathy in mice. *J. Am. Col. Cardiol.* 14:782.
26. Greenberg, S., J. El Khoury, F. di Virgilio, E.M. Kaplan, and S.C. Silverstein. 1991. Ca^{2+}-independent F-actin assembly and disassembly during Fc receptor–mediated phagocytosis in mouse macrophages. *J. Cell Biol.* 113:757.
27. Della Bianca, V., M. Grzeskowiak, and F. Rossi. 1990. Studies on molecular regulation of phagocytosis and activation of the NADPH oxidase in neutrophils. IgG and C3b-mediated ingestion and associated respiratory burst independent of phospholipid turnover and Ca^{2+} transients. *J. Immunol.* 144:1411.
28. Jaconi, M.E.E., D.P. Lew, J.L. Carpentier, K.E. Magnusson, M. Sjögren, and O. Stendahl. 1990. Cytosolic free calcium elevation mediates the phagosome-lysosome fusion during phagocytosis in human neutrophils. *J. Cell Biol.* 110:1555.
29. Bourne, H.R., D.A. Sanders, and F. McCormick. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature (Lond.).* 348:125.
30. Simon, M.I., P. Strathmann, and N. Gautam. 1991. Diversity of G proteins in signal transduction. *Science (Wash. DC).* 252: 802.
31. Kaziro, Y., H. Itoh, T. Kozasa, M. Nakafuku, and T. Satoh. 1991. Structure and function of signal-transducing GTP-binding proteins. *Annu. Rev. Biochem.* 60:349.
32. Hescheler, J., W. Rosenthal, K.-D. Hinsch, M. Wulfern, W. Trautwein, and G. Schultz. 1988. Angiotensin II–induced stimulation of voltage-dependent Ca^{2+} currents in an adrenal cortical cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:619.
33. Berridge, M.J. 1993. Inositol triphosphate and calcium signalling. *Nature (Lond.).* 361:315.
34. Stryer, L., and H.R. Bourne. 1986. G proteins: a family of signal transducers. *Annu. Rev. Cell Biol.* 2:391.
35. Pace, J., M.J. Hayman, and J. Galán. 1993. Signal transduction and invasion of epithelial cells by S. typhimurium. *Cell.* 72:505.
36. Fancis, C.L., T.A. Ryan, B.D. Jones, S.J. Smith, and S. Falkow. 1993. Ruffles induced by Salmonella and other stimuli direct macropinocytosis of bacteria. *Nature (Lond.).* 364:639.
37. Kao, J.P.Y., A.T. Harootunian, and R.Y. Tsien. 1989. Photochemically-generated cytosolic calcium pulses and their detection by fluo-3. *J. Biol. Chem.* 264:81779.