Physiological inducers. Eventually, the study of signal transduction in model systems has been extended to include the study of signal transduction in the mammalian host. The use of transgenic and null-mouse models has enabled the study of the role of specific signal transduction pathways in the context of the mammalian host. This has led to the identification of novel signal transduction pathways that are involved in the control of mammalian host responses to parasitism.

Responses to environmental changes are mediated by signalling pathways that coordinate processes involved in cell growth, development and function. Generally, in eukaryotes, this coordination is achieved by regulatory circuits that involve protein kinases and phosphatases, G proteins and second messengers. Changes in their activities can be initiated by external signals, environmental changes and internal homeostatic or cycling mechanisms, and in turn result in the modulation of the activities of these signals. The signals are integrated through specific receptors and intracellular signalling cascades, leading to the activation of downstream effectors that mediate the cellular response.

Plasmodium falciparum infection has been shown to result in the induction of cell death pathways in hepatocytes after injection with irradiated malaria sporozoites. This is mediated by the recruitment of host cell death receptors and the activation of caspases involved in cell death. This suggests that Plasmodium falciparum infection results in the induction of cell death pathways in hepatocytes that are not normally involved in cell death in the mammalian host.

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Unique characteristics of molecules that generate signals, their receptors, the downstream proteins or even the interactions of proteins in a pathway might be targeted in the design of new therapies. Much of the current research in parasites is predicated on the assumption that disruption of signal transduction, or induction of an inappropriate signal, would compromise the ability of parasites to survive in the mammalian host. This review will provide an overview of the potential mediators of responses to environmental changes that have been identified in trypanosomatids, and then discuss in more detail several of the second messenger systems that function in these parasites. Owing to space limitations, literature citations are representative rather than comprehensive.

Pathways and signalling molecules in trypanosomatids

Through gene discovery, molecules related to many of the key players in signal transduction in higher eukaryotes have been found in trypanosomatids (Table I). However, the emerging data suggest that the parasites might have a streamlined set of signalling molecules. Indeed, as discussed below and depicted in Fig. 1, the spectrum of molecules implicated in the receipt of extracellular signals in trypanosomatids is very limited, and the types of responses observed are also limited. Certain types of modular protein interaction domains are conspicuous in their absence from the gene databases (e.g. SRC homology regions 2 and 3 (SH2 and SH3 domains, respectively)). Many signalling pathways in other organisms culminate in the activation of transcription factors; such factors have not been identified in trypanosomatids. Because the major mechanisms for gene regulation in these parasites appear to be post-transcriptional, we speculate that signalling pathways regulating gene expression might target molecules regulating RNA processing or turnover. Many organisms induce signalling pathways in response to metabolites; whether trypanosomes do so is not yet known.

To date, studies of signal transduction in parasites have focused on differentiation, infectivity and cell growth. Unlike mammals, the Kinetoplastida have not been shown to display rapid, triggered changes in exocytosis, cell shape, transcription or metabolism. Little is known about the types of extracellular macromolecules that will alter kinetoplastid behaviour, or the type of response that is expected. The idea that specific molecules secreted by the host or parasite modulate parasite proliferation and development is very attractive. Among the molecules reported to have physiological effects on trypanosomatids are growth factors, cytokines and adrenergic ligands. One of the more interesting cases is that of interferon-γ (IFN-γ), which is reported to exert a growth regulatory effect on Trypanosoma brucei. Intriguingly, it also appears to modulate the activity of Kfr1, a protein kinase structurally related to mitogen-activated protein (MAP) kinase. Despite these provocative findings, most work on extracellular factors has not yet been validated by many other laboratories.

Trimeric G proteins function as molecular switches that can modulate downstream events during transmembrane signalling. Association of receptor with G protein initiates exchange of GDP for GTP by the α subunit and subsequent dissociation from the βγ dimer, leading to changes in interaction with other proteins and a signalling cascade. Leishmania, T. cruzi and T. brucei proteins have been identified that possess some or all of the following hallmarks of trimeric G proteins: (1) the ability to bind GTP; (2) an appropriate molecular weight; (3) crossreactivity with specific peptide antibodies; and (4) ADP-ribosylation in response to bacterial toxins that target G proteins in higher eukaryotes (reviewed in Ref. 10). It is unclear whether bacterial toxins can be used to manipulate G protein activity within intact trypanosomatids because these cells may lack surface receptors for the toxins. Perhaps the closest to a functional assay has been the demonstration that partially purified membrane proteins from T. cruzi inhibit glucagon stimulation of mammalian adenylate cyclase in a manner that can be reversed with pertussis toxin. These data are suggestive of Gs, although other inhibitory factors could have been involved. Overall, the ability of trypanosomatids to utilize trimeric G proteins in response to extracellular signals is not firmly established.

Table I. Trypanosomatid components potentially involved in environmental sensing

<table>
<thead>
<tr>
<th>Signal/sensing mechanism</th>
<th>Role in Kinetoplastids</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP regulators and effectors</td>
<td>Cell cycle control</td>
<td>3, 10, 11, 16, 27–30, 40, 50</td>
</tr>
<tr>
<td>Ca2+ regulators and effectors</td>
<td>Host cell invasion</td>
<td>10, 31–47</td>
</tr>
<tr>
<td>Inositol phosphates and phosphatidylinositol phosphates</td>
<td>Cell death</td>
<td></td>
</tr>
<tr>
<td>PH domains</td>
<td>Unknown pathways</td>
<td>37, 48–50, 52</td>
</tr>
<tr>
<td>Mosaic kinases and regulators</td>
<td>Cell-cycle control</td>
<td>11</td>
</tr>
<tr>
<td>Stage-regulated protein kinases</td>
<td>Unknown pathways</td>
<td>11, 17, 20</td>
</tr>
<tr>
<td>Phosphoproteins (kinase targets)</td>
<td>Absent: stimulus–response coupling?</td>
<td></td>
</tr>
<tr>
<td>Protein phosphatases</td>
<td>Unknown pathways</td>
<td></td>
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<tr>
<td>Heat-shock proteins</td>
<td>Cell differentiation</td>
<td>15, 18, 19, 24</td>
</tr>
<tr>
<td>Protein chaperones</td>
<td>Cell cycle control</td>
<td>21–23, 25</td>
</tr>
<tr>
<td></td>
<td>Unknown pathways</td>
<td>59</td>
</tr>
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</table>
the complexities of the cell cycle, as shown by the presence of genes encoding similar signalling proteins to those involved in the yeast and human cell cycle. Proteins that coordinate mitosis are present, including those with homology to cyclin-dependent and other cell cycle-regulated kinases, cyclins and other regulatory proteins11. Genes functioning in responses to environmental stresses, such as heat-shock proteins, are found in all organisms, including trypanosomatids. In higher eukaryotes, MAP kinase pathways regulate cell growth, apoptosis and stress responses. Genes encoding molecules with homology to protein kinases in the MAP kinase pathways have been identified in the trypanosomatids5,12 (accession numbers AF034925 and AF050218). Interestingly, certain environmental stresses, such as changes in temperature, pH or nutrients, act as triggers for the induction of developmental programmes. This convergence suggests that many of the same molecules might function in cell responses to stress, cell cycle control and differentiation. For example, in T. brucei growth arrest (and subsequent death) induced by treatment with concanavalin A and cell cycle arrest induced by development to stumpy forms are both marked by high expression of a transcript encoding an intracellular modulator of protein kinase C, PKA, cAMP-dependent protein kinase. PtdIns, phosphatidylinositol; SH2, SRC homology region 2. Dashed arrows and dark grey shading indicate missing components and connections; solid arrows and pale grey shading indicate known components and connections.

In contrast to these kinetic analyses, most studies of signalling molecules in development have focused on steady-state differences between developmental stages. There are numerous differences in the phosphoprotein profiles and protein kinase activities in the different developmental stages of trypanosomes14,15. In T. brucei, changes in adenylate cyclase activity are observed several hours after the shift16. In Leishmania, changes in protein phosphorylation have been observed within minutes of the environmental shift13 (D. Zilberstein, pers. commun.). Recent data indicate that, within 10 min after the shift to low temperature in the presence of cis-aconitate, trypanosomes commit to specific aspects of differentiation to procyclic forms14. In T. brucei, changes in adenylate cyclase activity are observed several hours after the shift16. Genes encoding several major classes of protein serine/threonine phosphatases have been cloned17–23. Although no genes encoding tyrosine kinases or phosphatases have been cloned, evidence for their activity has been obtained18–26. Also specified in trypanosomatid genomes are proteins participating in second messenger systems. These molecules include enzymes that regulate or are regulated by second messengers (see below).
Cyclic AMP

In one case, a conceptual link has been made between a ligand and a specific receptor-mediated signalling response. Bloodstream forms of *T. brucei* secrete a factor that stimulates the parasites to transform into non-dividing stumpy forms. Although the chemical nature of the stumpy-induction factor is not known, it appears to act through induction of CAMP formation. The action of this molecule can be mimicked by the addition of cAMP analogues. This dovetails nicely with the fact that the genes encoding receptor adenylate cyclases are the only known trypanosomatid genes encoding a receptor with a recognizable cytoplasmic signalling domain. These receptors appear to utilize CAMP as a second messenger. It is generally assumed that the receptor guanylate cyclases found in mammalian cells and contain a putative extracellular ligand-binding domain and a cytoplasmic adenylate cyclase domain. In both *Leishmania* and *T. brucei*, the genes exist as a multigene family, in which members differ significantly in the extracellular portion. These differences suggest that the proteins might interact with different ligands to modulate adenylate cyclase activity. However, like receptor guanylate cyclases, there is no evidence that activation of the trypanosomatid adenylate cyclases involves trimeric G proteins.

In a variety of laboratories suggests that CAMP might be important in regulating parasite development. As noted above, bursts of adenylate cyclase activity occur as *T. brucei* differentiates from bloodstream to procyclic form. The addition of non-hydrolysable CAMP analogues or stimulation of CAMP formation through phosphodiesterase activity can modulate development and proliferation of *T. cruzi*. Most CAMP-induced effects are mediated through protein kinase A. Genes encoding protein kinase A-related molecules have been identified in trypanosomatids. The targets of protein kinase A have not yet been identified in the parasites. Phosphodiesterases act to return CAMP levels to base-line. Although the enzymes have not yet been cloned in these organisms, the activity has been characterized.

Calcium

Ca2+ is universally recognized by all eukaryotic cells as a signalling molecule. Not surprisingly, kinetoplastids contain the biochemical machinery necessary to produce and terminate Ca2+ signals. In *T. brucei*, the nucleus acquires Ca2+ passively. In addition, all kinetoplastids examined to date contain three separate membrane compartments capable of unambiguously transporting Ca2+ in an energy-dependent manner, namely: the mitochondrion, plasma membrane and acidocalcisome. In addition, endoplasmic reticulum (ER) Ca2+ transport has been deduced from the cloning of genes encoding ER P-type Ca2+-ATPases. Variations in Ca2+ transport also has been detected, but has not been linked directly to the ER pool. Collectively, the redundant transporting organelles safeguard against uncontrolled changes in intracellular Ca2+ ([Ca2+]i). When multiple Ca2+-transporting organelles are disrupted with reactive oxygen species, the result for *T. brucei* is Ca2+-dependent fragmentation of nuclear DNA, loss of motility and cell death.

The acidosomes is perhaps the most distinctive organelle in the kinetoplastids. These organelles are of central importance to the regulation of cell responses in mammals. The role of the ER Ca2+ pool in trypanosomatids remains enigmatic. This problem arises because physiological activators capable of releasing unambiguously stored Ca2+ from the ER have not been found. The ER pool appears to be refractory to insulin (1,4,5)triphosphate ([Ins(1,4,5)P3], and perhaps thapsigargin). Organelar storage compartments are predicted to be of special significance for the propagation of Ca2+ signals in intracellular trypanosomatids, because the host cytoplasm contains very low levels of Ca2+. In *T. cruzi*, more Ca2+ is contained in amastigote acidosomes than in promastigotes, perhaps to accentuate the intracellular life style. By contrast, the extracellular stages have access to a large reservoir of extracellular Ca2+, which might be effective in regulating Ca2+ influx. Although the stimulatory molecules that might initiate a Ca2+ influx are not known, some progress has been made in elucidating the mechanism of influx in *T. brucei* by bypassing receptor function with amphiphilic peptides and amines. These molecules produce a specific Ca2+ influx in bloodstream and procyclic forms that appears to be mediated by activation of a cell-associated phospholipase A2 and concomitant release of arachidonic acid from membranes. The presence of this plasma membrane-associated pathway strongly suggests that extracellular triggers might modulate Ca2+ influx in these parasites. This mechanism of regulation clearly differs from most non-electrically coupled cells of the mammalian host, where phospholipase C and Ins(1,4,5)P3 are of central importance. The response of the cell to Ca2+ is determined by the cellular complement of Ca2+-binding proteins (CaBPs). Trypanosomatid contain a variety of CaBPs, including the universal regulatory protein calmodulin (CaM). To date, CaM dependency has been shown only for a limited number of target enzymes in trypanosomatids. These include cyclic nucleotide phosphodiesterase, CaM kinase II, nitric oxide synthase (reviewed in Ref. 32) and plasma membrane Ca2+-ATPase (reviewed in Ref. 32). In addition, the multifunctional elongation factor 1s binds CaM; a fact that has been confirmed for mammals and plants. Other CaBPs include isoforms of adenylate cyclase, a protein kinase A and endonuclease. A variety of putative CaBPs have also been cloned from exposed sequence tags (ESTs). Along with CaM, the flagellum of kinetoplastids contains one or more EF-hand CaBPs. The intriguing localization of these proteins to the flagellum suggests a potential role in motility or environmental sensing.

Specific processes regulated by Ca2+ flux and CaBPs have not been well characterized in trypanosomatids. In essence, the kinetoplastid Ca2+ pathway is the answer to a question that has yet to be posed and discovering the timing and the purpose of Ca2+ flux will be the next major challenge. A major unresolved aspect of Ca2+ research centres upon extracellular signals that might initiate Ca2+ flux and trigger the Ca2+ pathways. No soluble molecules, such as neurotransmitters or hormones, factors, have yet been found that affect [Ca2+]i. In *T. brucei*, a role for Ca2+ has been surmised for the invasion process in *T. cruzi*. In the amastigote, concentrations within the parasite and host have been reported. Contact is associated with Ca2+ flux in the...
Reviews

Parasite cytoplasm. The source of mobilized Ca$^{2+}$ has not been determined. However, the raised [Ca$^{2+}$] appears to be important for invasiveness because stabilization of [Ca$^{2+}$], with BAPTA or Quin-2 inhibits this process. Developmental changes and cell growth rate might also have Ca$^{2+}$-dependent components. Partial disruption of the CaM gene array in T. brucei produces a skew growth phenotype, and developmentally regulated CaM-binding proteins have been detected in T. cruzi and T. brucei. Changes in stored Ca$^{2+}$ also accompany the development process.

Overall, the kinetoplastid Ca$^{2+}$ system shares broad similarities with the mammalian host. However, differences in cell surface receptors, mechanism of channel regulation, homeostatic organelles and CaBP's indicate the presence of unique signalling components. How these components are coordinated to alter parasite behaviour is a key unresolved area for future research.

Phosphoinositides

Several inositol phosphates (InsPs) and phosphatidylinositol phosphates (PtdInsPs) play major roles as second messengers in signalling pathways. Analogous to the situation with Ca$^{2+}$, a complete InsP-PtdInsP signalling pathway would include triggered changes in InsP and PtdInsP levels, as well as proteins that respond to changes in the spectrum of these molecules. PtdInsPs are the lipid conjugate forms, which reside in membranes. Therefore, they are in an ideal location to respond rapidly to changes initiated at the plasma membrane. Phosphorylation of PtdInsPs is mediated by position-specific enzymes, including phosphatidylinositol 3-kinase (PI3K), which generates PtdIns(3,4,5)P$^3$, and PtdIns(3,4)P$^2$. InsPs are cytoplasmic molecules generated by cleavage of PtdInsP by phospholipase C; a gene encoding this enzyme has been identified in T. cruzi (accession numbers AB022677 and AF093565). This enzyme is distinct from glycosylphosphatidylinositol phospholipase C. In mammalian cells, PI3K and phospholipase C can be activated in response to lipid binding to cell surface receptors. Foetal calf serum and the cholinergic agent carbachol stimulate the production of inositol phosphates (InsPs) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P$^2$). PtdInsPs and InsPs have not been well studied in trypanosomatids. Their presence has been documented in bloodstream and procyclic forms of T. brucei, where the ratios of the specific forms differ. Ins(4,5)P$^2$, Ins(4,5,6)P$^3$, and Ins(1,4,5,6)P$^4$ have been identified in the parasites, with the concentrations of the last two being much higher in procyclic forms than in slender bloodstream forms. PtdInsPs have been studied in the epimastigote stage of T. cruzi. The parasites appeared to lack significant levels of PtdIns(3,4,5)P$^3$, although it should be noted that modifications at the 3 position (by PI3K) are usually induced by interactions with specific ligands. With respect to the enzymes of PtdIns metabo-lism, a gene encoding the C-terminal half of a putative PI3K has been cloned from T. brucei, suggesting that PtdIns phosphorylated at the 3 position could be present under certain conditions.

InsPs and PtdInsPs exert their effect by binding to and modulating protein function. As noted above, Ins(1,4,5,6)P$^4$ is a key effector of [Ca$^{2+}$], release in higher eukaryotes, but not in trypanosomes. A type of modular structure, termed the PH domain, interacts with PtdInsPs and InsPs. Different PH domains have different specificities for the various phosphorylated forms (see, for example, Ref. 54). The presence of specific InsPs or PtdInsPs can regulate the localization of PH-domain proteins to the membrane or cytoplasm. These second messengers can also regulate the activity of PH-domain proteins. PH domains have been identified on several protein kinases of trypanosomatids (in the databases), including one that is developmentally regulated in T. brucei. These findings raise the possibility that InsPs and phosphoinositides are important second messengers in these parasites.

Perspective

The examination of responses of parasites to environmental changes is one of the important contributions of our colleagues. The generation of this manuscript was supported in part by NIH AI24627 and by NIH AI24627.
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In this article, Bernardo Reina-San-Martín, Alain Cosson and Paola Minoprio summarize the marked alterations in the immune system functions after infection that might account for the poor success of effective parasite vaccine development. Many of the studies on oligoclonal B- and T-cell responses to parasite antigens aiming at vaccination strategies would seem to ignore more general, and perhaps fundamental, aspects of parasite-immune system interactions. In essence, because of its consequences on immunopathology and parasite escape, the authors ascribe a central importance in the pathogenesis of parasitic diseases to the 'non-specific' polyclonal lymphocyte activation that occurs during infection. Hence, novel targets and strategies for immune intervention should be considered.

It is a common belief that parasites, viruses, fungi and bacteria often evade the immune system through adaptive mechanisms, that render the immune response powerless. Classic approaches to vaccine development have focused on the study of specific, parasite-directed mechanisms such as 'immunodominant', 'immunopathological' and 'protective' epitopes, in search of molecules able to trigger protective immunity while avoiding evasion. However, there is an important gap in this thinking: the immunologically relevant interactions between the infectious agents and the host are not limited to specific immune responses. Parasites, viruses, fungi and bacteria can actually obliterate specific immune responses by simply triggering the machinery of polyclonal lymphocyte responses, thus resulting in a general lack of specificity of antibodies or T-cell responses to the microbial antigens during infection and in the immunosuppressive state that follows. This apparently reduced availability of lymphocyte clones able to respond to the infectious agent (and to heterologous antigens as well) can actually be explained either by conventional immunosuppression or by the extensive engagement of most lymphocyte populations in effector functions that are not clonally specific (hyperstimulation). The onset of autoimmunity, another unwanted and frequent consequence of infectious processes, can also be explained by the establishment of a long-lasting polyclonal activation, with the bulk of lymphocyte populations activated by the infection embodying host-directed cell clones involved in the evolution of self-aggressive mechanisms.

Vaccine strategies aimed at neutralization of mitogenic/superantigens and the control of non-specific responses are considered here; for additional references, see http://www.pasteur.fr/recherche/unites/trypano/minoprio/PTrefs.html.

Immune system-driven approach to infection

There are obvious difficulties faced by the immune system in order to eliminate a parasite. It is striking that a normal immune system is able promptly to reject tissues or organs that differ from the host by just a few amino acids in a single major histocompatibility complex (MHC) molecule. In contrast, the immune system is unable to eliminate parasites bearing a very complex and extremely different antigenic composition to that of the host. Nevertheless, the immune responses induced by parasite infections are sufficiently 'strong' to lead to progressive autoimmune pathologies that frequently can kill the host. Parasodically, immune mechanisms that are inappropriate to eliminate the parasite are capable of destroying the host itself.

Infectious agents share the ability to activate a high fraction of total lymphoid cells, many of which differentiate to exhibit effector functions. The consequences of this quasi-pandional activation of the immune system are: (1) the development of non-specific B- and T-cell responses, (2) the immunosuppression of humoral and cellular responses to homologous and heterologous antigens; and (3) the onset of autoimmune processes that might arise from the expansion of self-reactive clones. The magnitude of these responses and the profound perturbation of immune homeostasis they bring about are major hindrances to the development of effective vaccine strategies. Thus, lymphocyte activation in infection is essentially the result of mitogenic and/or superantigenic microbial components that elicit relatively poor specific responses. However, classic vaccination approaches have attempted to 'neutralize' 'immunogenic molecules' that do not prevent panchonal activation and are, therefore, ineffective in controlling mitogen-dependent immune disorders and parasite evasion.