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Metabolic flexibility in *Trypanosoma cruzi* amastigotes: Implications for persistence and drug sensitivity

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Abstract

Throughout their life cycle, parasitic organisms experience a variety of environmental conditions. To ensure persistence and transmission, some protozoan parasites are capable of adjusting their replication or converting to distinct life cycle stages. *Trypanosoma cruzi* is a 'generalist' parasite that is competent to infect various insect (triatomine) vectors and mammalian hosts. Within the mammalian host, *T. cruzi* replicates intracellularly as amastigotes and can persist for the lifetime of the host. The persistence of the parasites in tissues can lead to the development of Chagas disease. Recent work has identified growth plasticity and metabolic flexibility as aspects of amastigote biology that are important determinants of persistence in varied growth conditions and under drug pressure. A better understanding of the link between amastigote and host/tissue metabolism will aid in the development of new drugs or therapies that can limit disease pathology.

Keywords

metabolic flexibility; nutrient acquisition; T. cruzi; drug susceptibility; host-parasite

Introduction

Parasitic protozoans experience diversity in their environmental niches both within and between hosts. The variety of these environments includes variations in temperature, pH, oxygen saturation, nutrient availability, and immune activity. This heterogeneity can profoundly impact survival, metabolism [1], and the propensity to convert to distinct developmental stages to maximize transmission potential [2].

The kinetoplastid protozoan parasite *Trypanosoma cruzi* alternates between triatomine and mammalian hosts. *T. cruzi* can colonize a variety of mammalian species, earning its reputation as a 'generalist.' In humans, an infection can result in Chagas disease, which

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Declaration of interests

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most commonly manifests as cardiomyopathy. In the mammalian host, *T. cruzi* exists in two forms; the extracellular trypomastigote is motile and invades nucleated host cells followed by an escape from a transient parasitophorous vacuole and conversion to the intracellular amastigote form (Figure 1A). Amastigotes replicate by binary fission and can do so in most tissues [3]. Tissue damage and clinical disease are primarily dependent on the presence of amastigotes [4], and therefore therapies aim to eliminate these parasites. Following several rounds of replication, amastigotes convert to trypomastigotes and lyse the host cell to continue the cycle of infection. Parasitemia and tissue burden are high in the acute phase of the disease, but most immunocompetent individuals progress to a chronic stage characterized by low parasite levels without parasite eradication.

The mechanisms behind this remarkable persistence in diverse host environments, both between and within organisms, are still being explored, and their implications on drug efficacy are pertinent.

Metabolic flexibility and auxotrophy.

Parasites, by definition, are reliant on a host organism for growth and survival. The clearest examples of this dependence are instances where the parasite cannot synthesize a particular compound necessary for growth and instead have an absolute reliance on scavenging (i.e., auxotrophies). *T. cruzi* is auxotrophic for aromatic (phenylalanine, tryptophan, and tyrosine) and branched-chain acids (valine, leucine, and isoleucine) in addition to arginine, lysine, and histidine [5]. Similarly, *T. cruzi* is unable to synthesize heme [6], vitamins [7] and purines [8], making them reliant on salvage/transport pathways (Figure 1B). The free-living insect stage (epimastigote) has been commonly used to characterize these transport pathways, while less is known about the mechanisms of nutrient acquisition by the intracellular amastigote [5].

Conditions under which *T. cruzi* amastigotes retain the ability to synthesize and scavenge specific metabolites are less clear (Figure 1B). The evolutionary conservation of these anabolic pathways in the parasite may allow for metabolic flexibility that results in the ability of parasites to persist in a variety of environments. In this scenario, variability in amount, localization, or composition of scavenged metabolites is potentially balanced by *de novo* synthesis to maintain parasite viability, similar to other intracellular parasites [9–11]. Thus, the relative importance of specific host metabolic processes in *T. cruzi* amastigote replication is likely influenced by local environmental factors. These may include inherent metabolic differences in tissues colonized by the parasite [12] and arise with host immune responses that can control but not eliminate *T. cruzi* infection from the host.

Below, instances, where metabolites are synthesized and scavenged by *T. cruzi* are highlighted. We discuss the resulting implications for metabolic flexibility with a focus on the intracellular amastigote and its host.

Fatty Acids

Trypanosomes are capable of both scavenging fatty acids (FA) and *de novo* synthesis through a modular elongase (ELO) pathway to satisfy bulk fatty acid requirements,

including glycosylphosphatidylinositol production [11,13] in addition to maintaining components of a conventional type II FA synthase that is localized to the mitochondrion [14]. In *T. cruzi*, conversion to the intracellular, replicative amastigote is accompanied by a global transcriptomic shift in the parasite that includes upregulation of the ELO pathway transcripts [15]. Yet, the reduction of host FA metabolism or triacylglycerol (TAG) synthesis slows intracellular amastigote replication, suggesting that parasite *de novo* FA synthesis cannot wholly compensate for changes to the extracellular lipid environment [16]. These data indicate that the FA requirements of intracellular *T. cruzi* amastigotes are fulfilled through a combination of scavenging and synthesis, similar to that described for other protozoan parasites. Maintaining the ability for both synthesis and scavenging may allow for a degree of flexibility in changing host environmental conditions.

Sterols

Unlike *Plasmodium* and *Toxoplasma*, which are sterol auxotrophs [17,18], *T. cruzi* has maintained a pathway for *de novo* sterol synthesis in addition to scavenging cholesterol [19]. The products of these sterol synthesis pathways are ergostane-type sterols, distinct from host-derived cholesterol. Disruption of the first committed step of endogenous sterol synthesis (i.e., squalene synthase) leads to profound growth and morphological effects in both *T. cruzi* and *T. brucei* [20,21], suggesting the essentiality of these endogenous sterol products. Nevertheless, inhibition of sterol synthesis at points within the *de novo* synthesis pathway is tolerated by *Leishmania major*, a related kinetoplastid parasite, but is accompanied by a reduction in tolerance to various stressors [22,23]. *T. cruzi* enzymes within the *de novo* sterol synthesis pathway have been explored as drug targets (i.e., TcCYP51) due to their assumed essentiality [24]. However, the concept that endogenously derived sterols are important for parasite resilience raises the possibility that their essentiality is at least partially dependent on external factors or internal metabolic states [22].

Oxidative metabolism

T. cruzi amastigote replication occurs after escape from a parasitophorous vacuole, and consequently, during replication, amastigotes have direct access to a variety of potential nutrient sources. It has generally been assumed that in the host cytosol, amino acids and FAs provide the primary sources for energy production [25]. However, amastigotes are competent to utilize exogenous glucose, in addition to glutamine to support respiration [15,26]. Additionally, the maintenance of spare respiratory capacity by amastigotes predicts the ability of the parasite to flexibly meet changing energetic demands [15] like their host cells of residence. Overall, the ability of intracellular *T. cruzi* amastigotes to utilize multiple fuel sources likely re-enforces their ability to persist in a variety of dynamic environments. Inhibition of amastigote mitochondrial respiration slows but does not prohibit replication [27], even though the capacity for β-oxidation, as inferred from enoyl-CoA hydratase knockout parasites, is important for amastigote replication [28]. These studies demonstrate that amastigote metabolic flexibility includes the ability to rely on non-respiratory carbon sources.

Identifying the relative importance of scavenged metabolites to intracellular *T. cruzi* amastigote metabolism is particularly challenging because the host cell remains metabolically active as parasites divide. As such, the host cell can readily metabolize metabolic tracers before reaching amastigotes, complicating the interpretation of metabolic data, particularly for the analysis of metabolites that are rapidly turned over. While the ultimate goal is to understand amastigote metabolism inside host cells, several approaches directly study amastigotes. Axenically derived amastigotes can be generated from trypomastigotes but have distinct growth, morphological and biochemical characteristics that differentiate them from intracellular amastigotes [29–31]. Alternatively, amastigotes directly isolated from infected cultures are metabolically active but have limited replicative capacity. The development of protocols to isolate and profile the metabolites directly from intracellular amastigotes should aim to minimize host contamination while being rapid to perform to effectively quench parasite metabolism to limit isolation-related metabolic shifts [32]. Direct measurement of metabolites from isolated amastigotes will be essential to validate the metabolic networks that are predicted from genomic and transcriptional data [15,33].

Sensing and responding to external cues.

Metabolites

Parasites can sense, either directly or indirectly, and adapt to changes in their environment to ensure persistence or growth within a host while optimizing the potential for transmission to a new host. Failure to regulate nutrient uptake in nutrient-rich environments [34] or adjust proliferation rates in nutrient limiting conditions [35] can result in parasite elimination. *T. cruzi* amastigotes are able to dynamically regulate their cell cycle by extending the G1 phase in response to shifting metabolic environments [27]. *T. cruzi* can also regulate the import of molecules such as heme that are necessary for replication but can be toxic when in excess [6]. While the sensing mechanisms of these processes are unknown, other parasites are capable of monitoring their intracellular metabolic states [35] and use external sensors (e.g., flagella) to probe their immediate environment [36,37]. Intriguingly, in addition to the predicted presence of intracellular sensors such as AMP-activated protein kinases [38], the short flagellum of intracellular *T. cruzi* amastigotes establishes close contact with host mitochondria and may provide a mechanism by which the parasite can gauge the immediate intracellular environment of the host cell [39].

Immune Responses

Infection with *T. cruzi* is followed by a brief incubation period and subsequent progression to the acute phase of the disease characterized by microscopically detectable parasitemia and nonspecific symptoms. Most infected individuals survive the acute phase and progress to a chronic stage where parasitemia is intermittently detectable by PCR. The majority of our understanding regarding the dynamics and localization of amastigotes in the chronic phase comes from mouse models of infection. In a murine host, *T. cruzi* can persist at low levels [40] in the gut, accompanied by scarce focal growth in other tissues [3,41]. During chronic infection, amastigotes remain replicative but have dramatically shifted cell cycle [42]. The specific determinants behind this process have not been characterized, but the induction

of self-limiting replication to ensure protection from the immune response is not without precedence. For instance, *Leishmania* parasites induce a "stringent response" in tissue lesions, limiting parasite growth presumably to limit macrophage activation [43,44]. In addition, *Leishmania* parasites in chronic lesions do not natively utilize all nutrient sources available. Instead, they are primed to catabolize nutrients that have limited ROS production and therefore allow for persistence *in vivo* [45], highlighting the link between metabolic state and immune evasion. While little is known about switches in *T. cruzi* amastigote metabolism during chronic infection, localized alterations to the host metabolome and microbiome have potential implications for pathogenesis [12].

Implications of metabolic heterogeneity and flexibility for drug development.

Benznidazole remains the standard for the treatment of *T. cruzi* infection. Treatment during the acute phase appears more efficacious than in the chronic phase, where baseline cardiac function and age may influence clinical benefit [46]. However, following treatment, a proportion of patients continue to harbor parasites, suggesting that parasites can withstand treatment or that benznidazole does not reach a proportion of tissue-resident parasites [47,48]. In response to non-lethal amounts of benznidazole *in vitro,* the amastigote cell cycle shifts towards a higher proportion of G1 parasites in a dose-dependent manner. This response is consistent with a DNA damage-based mechanism [49] of action followed by re-entry into the cell cycle at a rate inversely proportional to drug concentration [27]. By a similar mechanism, amastigotes tolerate inhibition of cytochrome b through a dramatically slowed cell cycle and have a rapid resumption of growth following the release of inhibition [27]. Combined, these observations suggest that the inherent ability of intracellular *T. cruzi* amastigotes to sense and respond through cell cycle plasticity should be taken into consideration when evaluating the effects of drug treatment.

Drug screening traditionally employs nutritionally rich grow media and evaluates compound efficacies based on a failure of pathogen growth. While the metabolic state and growth rate of microorganisms is challenging to uncouple, in bacteria, the metabolic state has been shown to impact antibiotic efficacy [50]. Similarly, clinically relevant mutations in *E. coli* central metabolic genes, as opposed to mutations in the targeted pathway, can alter basal respiration and modulate drug activity [51]. The diversity of *in vivo* environmental niches coupled with metabolic and growth flexibility of parasitic organisms can complicate the utility of pharmacotherapies. For instance, Inhibitors of endogenous ergostane-type sterol biosynthesis (i.e., azoles) showed promising preclinical activity, but in contrast to benznidazole, a rapid rebound of parasitemia followed the cessation of therapy [47]. *In vitro*, the metabolic environment, particularly the amount of available glutamine (Figure 2), determines azole efficacy against *T. cruzi* amastigotes [52]. Interestingly, in the *in vivo* mouse model of chronic infection and under azole treatment, amastigotes persist in the large intestine, a site of reduced glutamine availability [53]. These data suggest that amastigote metabolic flexibility and the diversity of host environments can potentially result in infections that are recalcitrant to specific treatments due to variations in metabolic state.

Conclusions:

Trypanosoma cruzi is a generalist parasite both in the host range and within-host distribution. While such flexibility is likely accomplished through the ability of these parasites to sense and respond to environmental perturbations via direct or indirect mechanisms, little is known about this capability. One crucial consequence of adaptability is that anti-parasitic drugs that induce metabolic changes in *T. cruzi* amastigotes can be contextually efficacious due to the parasite's capacity to exist in various metabolic states with the potential to confound new compound screening efforts. Future research will focus on understanding how amastigote metabolism allows for growth in diverse environments and unravel the processes by which amastigotes sense and respond to their environment. Understanding the link between amastigote metabolism and the resident host cell/tissue will aid the evaluation of candidate pharmacotherapies with the ultimate goal of disrupting processes integral to parasite persistence independent of their environment.

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Highlights

- *T. cruzi* amastigotes can dynamically respond to changes in their environment
- Growth and metabolic flexibility allow for *T. cruzi* growth in diverse environments
- Mechanisms of *T. cruzi* growth plasticity have implications for drug development

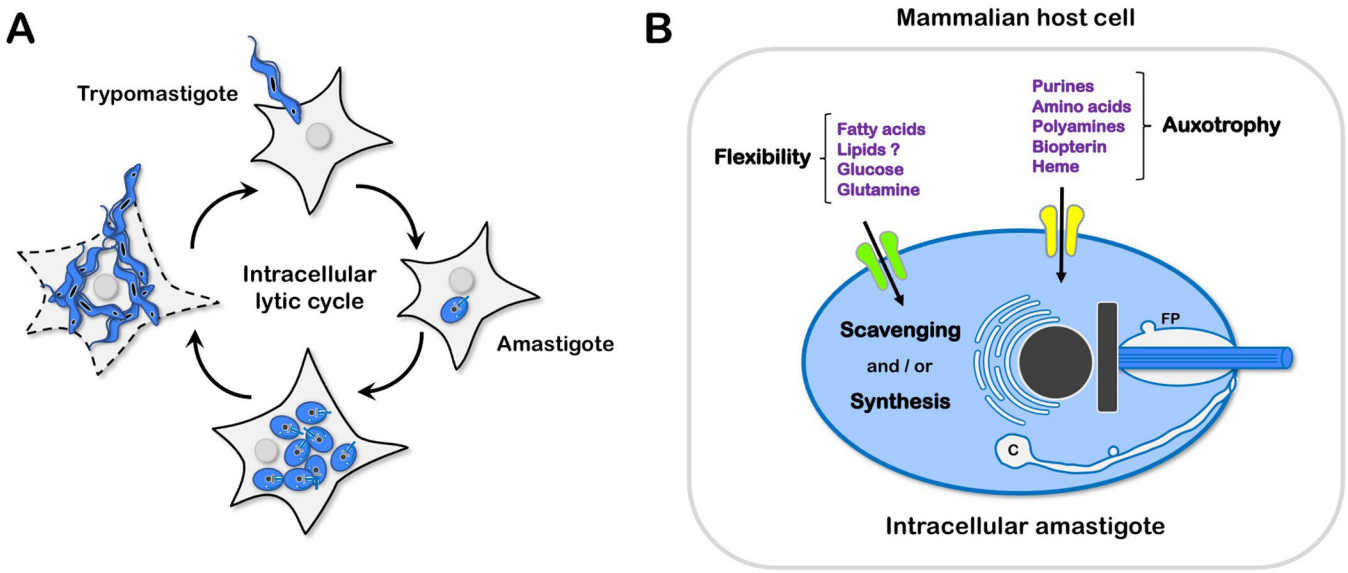

Figure 1.

(A) *Trypanosoma cruzi* infection cycle in mammalian host cells. Motile *T. cruzi* trypomastigotes actively invade nucleated mammalian cells to establish intracellular infection. Host cell entry triggers a developmental program [15], resulting in the formation of intracellular amastigotes that coincides with the localization of the parasite in the host cell cytoplasm. The timing of this process varies with parasite strain and host cell type. Replication competent amastigotes are typically formed by 16–20 hours post-infection (hpi) and begin to proliferate at ~24 hpi [27]. After several rounds of replication and cell division, intracellular amastigotes cease division and differentiate to trypomastigotes that eventually egress from the host cell where they can invade other cells. **(B) Interaction of intracellular *T. cruzi* amastigotes with host nutrient sources.** Generalized model depicting the transport of nutrients for which amastigotes have strict dependence on the host cell (auxotrophy) and those that can be acquired and synthesized by the parasite (flexibility). Transporters are drawn on the plasma membrane for simplicity but can be distributed within the flagellar pocket (FP) and cytostome/cytopharynx (C), and other endocytic organelles.

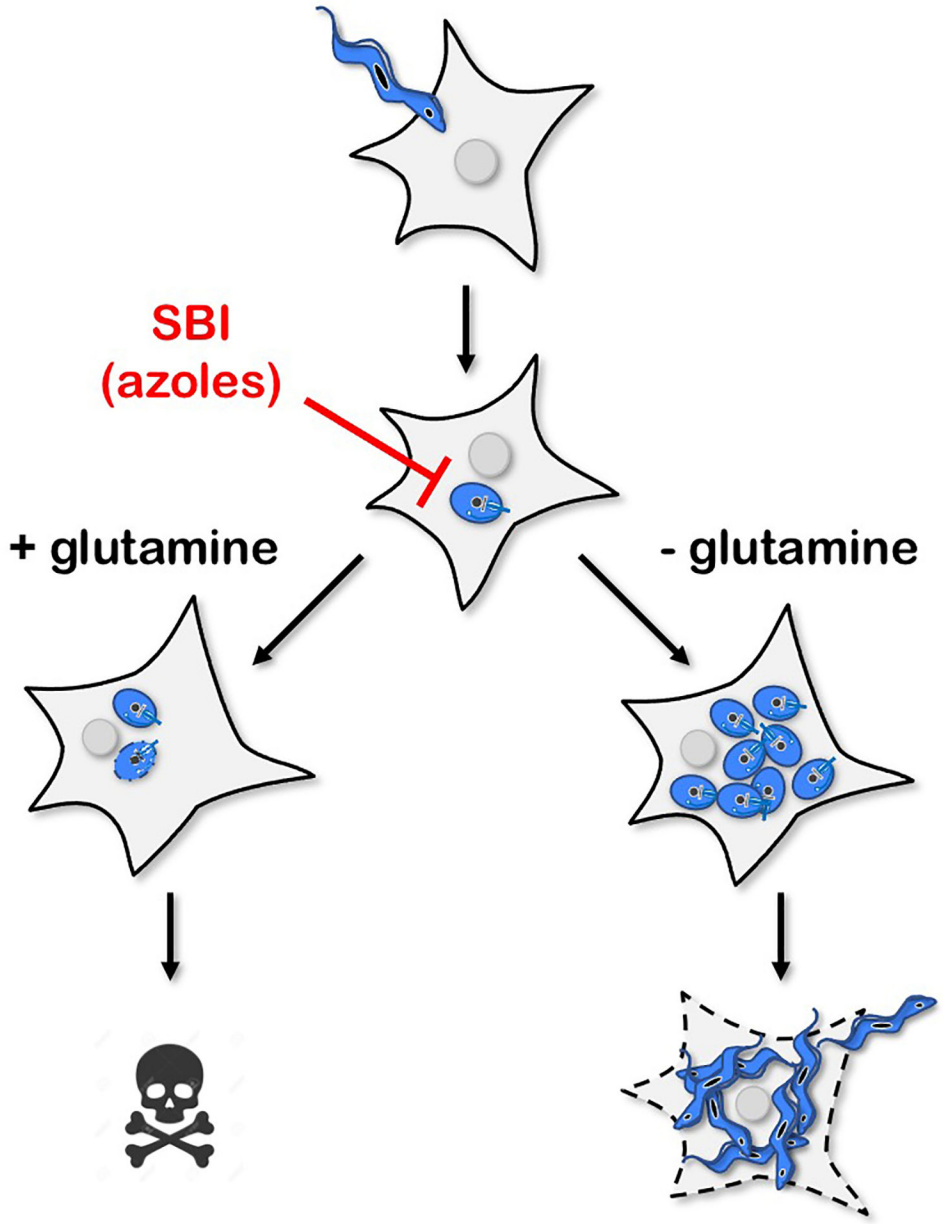

Figure 2. Schematic illustrating the impact of environmental heterogeneity on the susceptibility of intracellular *T. cruzi* amastigotes to azole inhibitors of parasite sterol biosynthesis.

In cell culture models, a single change in the composition of the medium (the presence or absence of supplemental glutamine) alters the outcome of *T. cruzi* infection following exposure to lethal concentrations of azoles [52]. In the presence of supplemental glutamine, amastigotes succumb to azoles, whereas glutamine restriction is associated with parasite protection at the population level.