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Current Opinion in Microbiology

Developmental differentiation in *Leishmania* lifecycle progression: post-transcriptional control conducts the orchestra

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The successful progression of *Leishmania* spp. through their lifecycle entails a series of differentiation processes; the proliferative procyclic promastigote forms become quiescent, human-infective metacyclic promastigotes during metacyclogenesis in the sandfly vector, which then differentiate into amastigotes during amastigogenesis in the mammalian host. The progression to these infective forms requires two components: environmental cues and a coordinated cellular response. Recent studies have shown that the *Leishmania* cellular transformation into mammalian-infective stages is triggered by broad changes in the absolute and relative RNA and protein levels. In this review, we will discuss the implications of *Leishmania* transcriptomic and proteomic fluctuations, which adapt the parasitic cell for survival.

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Current Opinion in Microbiology 2016, **34**:82–89

This review comes from a themed issue on **Fungi and parasitic diseases**

Edited by **Gero Steinberg**

http://dx.doi.org/10.1016/j.mib.2016.08.004

Introduction

Leishmania spp. are protozoan parasites which belong to the order Kinetoplastida and are the aetiological agents of the human leishmaniases. These diseases cause 20 000–40 000 deaths per year and threaten 310 million people worldwide [1]. *Leishmania* protozoan flagellates have evolved from an ancestral monoxenous life in insects to a complex dixenous parasitic lifecycle which alternates between a sandfly vector and mammalian hosts [2,3]. As a key driver for this evolutionary adaptation, *Leishmania* parasites remodel their cellular architecture and physiological properties for survival through stressful environmental conditions inside and out of the insect alimentary tract. Specific developmental adaptations have shaped the appearance of transmission-optimised metacyclic promastigote forms in the insect vector and intracellular amastigote forms in mammalian leukocytes. These two consecutive differentiation processes are called metacyclogenesis, and amastigogenesis (Figure 1).

An unusual characteristic of kinetoplastid cells is the near absence of transcriptional control. Genes are arranged and transcribed into long polycistronic transcription units (PTUs) and, in the context of the *Leishmania* spp. genomes, subjected to a high genomic plasticity which results in frequent aneuploidies and copy number variations [4,5]. Once transcribed, the mRNA is matured by *trans*-splicing of a capped Spliced Leader sequence (SL) to the 5′ end of all mRNAs coupled to 3′ cleavage and polyadenylation in order to be targeted for nuclear export [6–8]. Reduced transcriptional regulation makes these organisms extreme models for studying post-transcriptional and post-translational processes in cell development.

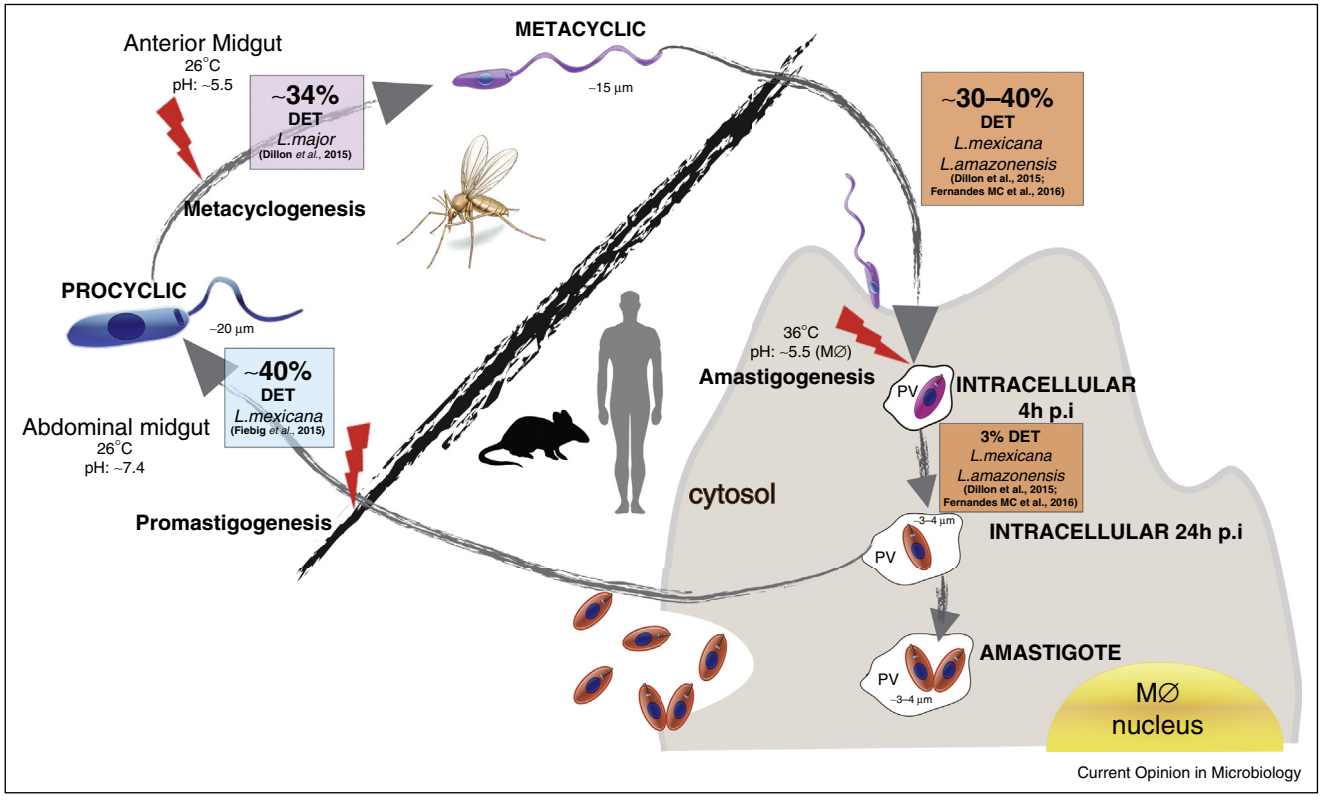
In prokaryotic and eukaryotic organisms post-transcriptional and post-translational networks enable immediate response and survival during environmental stress [9–18]. Examples of this include small molecules termed riboswitches, that bind regulatory elements in mRNA *UTRs* and target them for swift degradation, or subcellular storage granules containing translationally arrested RNAs that form in response to specific conditions in many cell types [18–20].

The main differentiation processes within the *Leishmania* parasite lifecycle can be replicated in culture to a degree. This convenience makes the *Leishmania* parasite a good model system to study developmental processes in infectious disease. In this review, we will discuss recent reports highlighting the dynamic nature of RNA and its translation into protein during differentiation in *Leishmania* spp. parasites.

RNA and protein changes during metacyclogenesis

Metacyclogenesis is the developmental process undertaken by leptomonad forms once they reach the anterior midgut of an infected sandfly (Figure 1). Metacyclic promastigotes are defined as mammalian infective, highly motile, non-replicative cells, characterized by a large

Figure 1



Gene expression changes during the *Leishmania* life cycle. The lifecycle begins when an infected female phlebotomine sand fly bites the skin of a mammalian host and transmits infective metacyclic promastigote forms. These are phagocytized by macrophages and other mononuclear phagocytic cells. Inside the host cell, the parasite undergoes a differentiation process (amastigogenesis) which gives rise to rounded amastigotes that multiply inside a parasitophorous vacuole (PV) and may infect other phagocytic cells after host cell death. Amastigotes are taken up during the bloodmeal of the sandfly and transform in the midgut into procyclic promastigotes which multiply by binary fission. Procyclic-derived forms will finally migrate towards the anterior midgut where they differentiate into metacyclic forms (metacyclogenesis) which may then infect another mammalian host. DET: differentially expressed transcripts. MØ: macrophage. For the purpose of simplicity, we display the example of *L. mexicana* amastigote growth, which is within one PV for each host cell.

flagellum at least twice the length of the slender cell body (~8 µm × 1.2–1.5 µm) [21,22]. Another defining characteristic is that the major promastigote-stage parasite surface glycoconjugate lipophosphoglycan (LPG), composed of repeating [Gal-Man-PO4] units [23] is extended in this stage; involving the elongation of the polysaccharide portion of the molecule [24].

To date, a relatively high number of factors including low pH (~5.5), anaerobic conditions, nutrient starvation, cell density (10^{10}–10^{11} leptomonads/ml), absence of purines and decline in tetrahydrobiopterine levels have been identified *in vitro* as triggers for metacyclogenesis [25–30]. Although their precise coordination for inducing metacyclogenesis *in vivo* is not known, this suggests that differentiation in the anterior midgut is driven by the combination of several cues with some potential redundancy.

During differentiation, the metacyclic promastigotes accumulate in an environment dominated by a filamentous phosphoglycan structure secreted by proximal leptomonad forms known as the Promastigote Secretory Gel (PSG) plug [31••,32]. This dense matrix blocks the anterior midgut and hinders the sandfly stomodeal valve function, thus promoting the reflux of parasites during the bloodmeal [22,31••]. The high motility and small dimensions of metacyclic promastigote forms promote the discrete selection of these cells to navigate through the PSG. Interestingly, it has been suggested that the production of the PSG might also act as a trigger for metacyclogenesis [22,31••]. As revealed by *in situ* metabolic labeling of *L. mexicana* cells, the metacyclic promastigote forms are characterized by a 4–6-fold reduction of RNA, protein and lipid total turnover compared to the highly replicative procyclic promastigote stage [33••]. These data reflect the reduction of the total cell volume and the quiescent status of metacyclic promastigote cells. Similarly, RNA-seq analysis has recently shown that RNAs change in both absolute and relative levels compared to procyclic promastigote stage parasites [34••].