Vacuolar type H^+ ATPase: a master regulator of stress responses in *Leishmania*

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Leishmania parasites alternate between two main life forms in two different hosts: the promastigotes in the sandfly vector and the amastigotes in the macrophages of the mammalian host. During this life cycle, they need to adapt to many environmental changes, including pH and temperature. A systematic CRISPR/Cas9 gene deletion screen of *L. mexicana* transporter proteins revealed that while the vacuolar-type H^+ ATPase (v-ATPase) is dispensable for the growth of promastigote form parasites *in vitro* under standard culture conditions, it is required for survival in the mammalian host, colonization of the sandfly vector and metacyclogenesis (Albuquerque-Wendt et al., 2025; Sadlova et al., 2026). The v-ATPase is a highly conserved, multi subunit proton pump, which canonically regulates the luminal pH of a variety of organelles, such as lysosomes, endosomes and acidocalcisomes. While v-ATPases have been extensively studied in mammalian and yeast systems, its physiological role in *Leishmania* remains poorly defined.

In this study, we aimed to

1) Explore the conditions under which v-ATPase is required for parasite growth and fitness
2) Investigate the role of v-ATPase in *L. mexicana* autophagy

To address aim (1) we assessed the growth of v-ATPase knockout (KO), addback (AB) and parental promastigotes in different conditions *in vitro*, namely pH 5.5 and pH 8.45 at 27 ºC

temperature, pH 7.4 and pH 5.5 at 34 ºC, and hyperosmotic stress. The v-ATPase KO parasites were more sensitive to all tested conditions than the parental and AB controls, and non-viable at pH 5.5 at 34 ºC. This suggests that the v-ATPase is essential for adaptation to a variety of different environmental conditions that are faced by the parasites in their life cycle.

Examination of cells subjected to these stress conditions by live cell imaging and transmission electron microscopy showed that v-ATPase KO parasites formed enlarged vacuolar structures when kept in dense *in vitro* cultures, under hyperosmotic stress, or exposed to heat. Endogenously tagged autophagosome marker protein ATG8 and lysosome marker cysteine peptidase A (CPA) both localized to these enlarged vacuolar structures. This indicated that the v-ATPase KO parasites were arrested at the final stage of autophagy, which is autolysosomal degradation of cellular waste material by lysosomal hydrolases. To test if this is due to a change in the luminal pH of the lysosome in the v-ATPase KOs, we measured this with a genetically encoded, ratiometric pH biosensor pHLuorin2. In parental cells lysosome had a luminal pH of 5.6. Upon perturbations of v-ATPase function by genetic deletion of one of its subunits, or by pharmacological inhibition with Bafilomycin A1, the lysosomal pH was 6.6-7.2, implicating the v-ATPase in the acidification of the *Leishmania* lysosome and regulation of autophagy.

Given the essential roles of the v-ATPase in *L. mexicana* cell biology, and the tractability of this system to study essential subunits, this work highlights the v-ATPase as a potential drug target.

References

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