Protein turnover as a key determinant in leishmania donovani parasite stage differentiation

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Leishmania survival and pathogenicity depends on the parasite's capacity to adapt to different host environments through stage differentiation of promastigotes within the sand fly, and of amastigotes inside mammalian host cells. *Leishmania* stage-specific expression occurs in the absence of classical transcriptional regulation, raising the question on alternative regulatory mechanisms. We investigated these mechanisms applying RNAseq, label-free quantitative proteomics and phosphoproteomics approaches on hamster-purified amastigotes and corresponding, culture-derived promastigotes. Comparison of the stage-specific transcriptomes and proteomes revealed a three times higher dynamic range for protein compared to RNA abundance suggesting that translational and post-translational mechanisms may outweigh RNA turnover in regulating stage differentiation. We next investigated protein turnover by applying label-free quantitative proteomic on both amastigotes and promastigotes in presence or absence of the irreversible, proteasomal inhibitor lactacystin. Inhibitor-treated amastigotes were viable but failed to convert into promastigotes in culture, revealing an essential role of protein degradation in *Leishmania* development. We identified 180 proteins (fold change ≥ 2, adj. p-value < 0.01) as proteasomal targets during the amastigote-to-promastigote transition, which represent putative differentiation factors. Applied on promastigotes, lactacystin treatment rescued 289 proteins from degradation (fold change ≥ 2, adj. p-value < 0.01) but neither affected parasite morphology nor proliferation. Interestingly, we observed stabilization of amastigote-specific proteins in lactacystin-treated promastigotes (and vice versa) suggesting a role of proteasomal degradation in regulating stage-specific protein abundance. Surprisingly, 18 proteins (fold change ≥ 2, adj. p-value < 0.01) were stabilized in both stages, including 11 proteins that were only identified in lactacystin treated parasites, thus uncovering a set of proteins that undergo constitutive degraded in our experimental system. Our data identified respectively 6 and 11 protein kinases that were rescued from degradation in treated amastigotes and promastigotes, suggesting differential protein kinase turnover as a regulatory switch in parasite development. Finally, we investigated the pathways controlled by protein kinase activities during differentiation using label-free, quantitative phospho-proteomics analysis of splenic amastigotes and culture-derived promastigotes. We identified 7095 phosphopeptides in promastigotes and 2080 in amastigotes of which 6128 (61%) are exclusive to one stage or the other. Twenty five proteins with exclusive stage-specific phosphorylation were linked to proteasomal protein degradation, including 3 proteasomal subunits, 5 ubiquitin transferases, 5 ubiquitin ligases, 1 ubiquitin-conjugating enzyme, 1 ubiquitin-activating enzyme and 10 ubiquitin hydrolases. In conclusion, our results link stage-specific, proteasomal degradation of protein kinases to parasite differentiation, and vice versa link stage-specific protein kinase activities to differential phosphorylation of proteasomal components. This reciprocal relationship likely establishes a proteasome/kinome regulatory

network that controls *Leishmania* stage differentiation and confirms both the kinome and the proteasome as interesting targets for anti-parasitic intervention.

Keywords: *LEISHMANIA DONOVANI*; DIFFERENTIATION; PROTEIN TURNOVER; KINASE; PROTEASOME

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