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Biochemical Characterization and Subcellular Localization of the Tyrosine and Phosphoinositide Dual Specificity Phosphatase LdPIP22, a potential drug target from Leishmania donovani

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Modulation of phosphoinositide (PI) metabolism is important for the pathogenicity and virulence of many human pathogens. While, PI phosphatases from bacterial pathogens that modulate PI metabolism in the host constitute already attractive targets for chemotherapy [1], this aspect is poorly explored for intracellular protozoan parasites like *Leishmania*. The kinetoplastids' phosphatome contains a large number of atypical dual-specificity protein phosphatases (aDSP) [2]. A subgroup of aDSPs, designated as atypical lipid phosphatases (ALPs) from *Leishmania* parasites with no human homologues, share similar catalytic P-loop motif to the *Mycobacterium tuberculosis* virulence factor MptpB PI phosphatase [2,3]. This work investigates the biochemical properties and subcellular localization of an ALP family member, the *Ld*PIP22 [LDBPK_220120], from the visceral leishmaniasis causative agent *Leishmania donovani*, as a potential drug target. The bacterially produced rN15-*Ld*PIP22-His (with an additional 15-peptide at the N-terminus and a 8His-tag at the C-terminus) dephosphorylates the pNPP substrate with optimum pH=6. The Km and Vmax values of this recombinant enzyme were determined and it was further shown that the rN15-*Ld*PIP22-His dephosphorylates phospo-Tyrosine peptides and PI3P and PI4P phosphoinositides. Biochemically, the parasitic endogenous *Ld*PIP22 protein was detected in *Leishmania* protein fractions enriched in membranes. Additionally we have evidence that it is secreted in the parasites' extracellular medium. Furthermore, using confocal microscopy we showed that recombinant *Ld*PIP22-GFP expressed in mammalian cells localizes on plasma membrane sites of dynamic actin polymerization where PIs play important role, while the bacterial rN15-*Ld*PIP22-His seems to interact with low affinity with F-actin *in vitro*. Finally, super resolution (STED) microscopy showed partial colocalization of native *Ld*PIP22 epitopes and parasitic actin in *Leishmania* cells. Generation of *L. donovani* overexpressing r*Ld*PIP22-6His parasites is in progress for isolation of the enzyme produced in the *Leishmania* cells for structural analysis and study of its possible role in the parasite's life and virulence.

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Substrate specificity properties of the xanthine, guanine, and uracil transporters of the Acinetobacter calcoaceticus – Acinetobacter baumanii complex

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To overcome the well-known resistance of pathogens such as *Acinetobacter baumanii* to existing antibiotics, alternative antimicrobial approaches are needed. One approach might involve exploitation of key metabolite transport systems to target pathogens with unique anti-metabolite analogs. In particular, using nucleobase transporters as pathogen targets, offers the advantage that homologs of the relevant uptake systems are absent or related with different specificity in the human genome. The putative nucleobase-transporter genes of *Acinetobacter calcoaceticus – Acinetobacter baumanii* complex belong to families Nucleobase:Cation Symporter-1 (NCS1) or NCS2. These genes were mobilized from *A. calcoaceticus* and analyzed functionally in *Escherichia coli* K-12. They encode high-affinity NCS2 transporters for xanthine (AcS6Q3), xanthine/uric acid (AcS4X6), uracil (AcS572), or guanine (AcRZR2) and a NCS1 transporter for uracil/guanine (AcS356). When compared with non-pathogenic homologs, the specificity data reveal novel profiles. AcS4X6 recognizes xanthine, 8-methylxanthine, oxypurinol, uric acid, with high affinity, and 2-thioxanthine, 6-thioxanthine, 7-methylxanthine, with low affinity; its closest homolog, rhizobial SmLL9 (69% identity), recognizes all these compounds with high affinity. In an attempt to rationalize the difference, we subjected AcS4X6 to mutagenesis at putatively contributory residues and showed that mutant V272I/M302T/L389I/A391F/C421A (replacements distant from the homology-modeled binding site) emulates the profile of SmLL9. Secondly, AcS572 is strictly specific for uracil although it shares 68% identity with the broad-specificity uracil/thymine/xanthine transporter RutG of *E. coli*. AcS572 differs from RutG in two peripheral binding-site residues (Phe-87/Ala, Gly-308/Ala), which might account for the inability to recognize thymine or xanthine. Finally, uniquely in NCS2, AcRZR2 is strictly specific for guanine and does not transport adenine or hypoxanthine; its closest homologs are transporters for adenine/guanine/hypoxanthine (SmVC3, 56% identity).