

Analysis of Nucleobases, Nucleotides and cyclic Nucleotides in *Leishmania*

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Nucleotides are core macromolecules of bioenergetics, redox processes, nucleic acids metabolism, and cell signalling. Kinetoplastid parasites are reliant on salvage pathways to fulfil their requirements of purine and pyrimidine derived nucleotides. These protozoa have also evolved to uptake nucleobases and nucleosides from the extracellular milieu at their distinct host environments. Nucleotide biosynthesis requires a nucleobase linked to a pentose sugar (nucleoside) that can thus be mono, di or triphosphorylated (nucleotides) harbouring high-energy phosphoanhydride bonds. This latter can also give rise to cyclic nucleotides such as cAMP and the magic spot nucleotide pppGpp (guanosine pentaphosphate). Here, we used an HPLC-based method enabling the identification of the most abundant nucleobases, purine and pyrimidine nucleotides, and cAMP in promastigote forms of *Leishmania mexicana*. A pppGpp synthase identified in the free-living protist *Bodo saltans* was expressed in *L. mexicana* and its metabolic product was also detected using this method. The role of inositol pyrophosphate (5-InsP₇) as a key metabolite controlling the pool of purine nucleotides in these organisms will also be discussed. This method can be useful to analyse the nucleotide response to pharmacological or genetic perturbations in different cell systems.

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