Leishmania mexicana protein kinase interactions

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Our lab has a long-standing interest in mitogen-activated protein (MAP) kinases and their signalling pathways. Improved proximity-labelling systems together with CRISPR-Cas9 in *Leishmania mexicana* as well as the success of AlphaFold to model interacting proteins allowed to identify interacting protein kinases. We used a putative MAP kinase kinase kinase (LmxM.19.0150) to identify its potential interaction partners employing a two-component biotinylation system with miniTurbo as the biotin ligase. Among other proteins, this returned a list of 21 protein kinases. LmxM.19.0150 showed close interaction with the putative Ca^{2+}/calmodulin-dependent protein kinase LmxM.07.0900 in AlphaFold. Using CRISPR-Cas9 *Leishmania* cell lines expressing LmxM.19.0150-mNG and LmxM.07.0900-mRFP were generated to reveal fluorescent signal co-localisation in promastigotes. When co-expressed in *Escherichia coli* using untagged LmxM.19.0150 with hexahistidine-tagged LmxM.07.0900, bands of different mobility in SDS-PAGE were observed for the purified 6His-LmxM.07.0900 compared to expression of 6His-LmxM.07.0900 alone. Phosphorylation site analysis using tandem mass spectrometry showed differences in the phosphorylation pattern in the samples. Moreover, LmxM.19.0150 co-purified along with 6His-LmxM.07.0900 using a Co^{2+}-nitrilotriacetic acid resin suggesting a close interaction of the protein kinases. LmxM.18.0640 was found to be highly homologous to LmxM.07.0900 suggesting that this kinase might be able to interact as well. Co-localisation of LmxM.07.0900-mRFP with LmxM.18.0640-mNG could be demonstrated using fluorescence microscopy. Co-expression of combinations of the three protein kinases in *E. coli* followed by sequential purification via glutathione-S-transferase-tag and 6His-tag suggests interaction of the kinases in this heterologous system. Alphafold modelling appears to support this interaction.