**Defining the molecular determinants required for Leishmania life cycle progression and virulence.**

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Differentiation between distinct stages is fundamental for the life cycle of intracellular protozoan parasites and for transmission between hosts, requiring stringent spatial and temporal regulation. We applied kinome-wide gene deletion and gene tagging in *Leishmania mexicana* promastigotes to define protein kinases with life cycle transition roles. Phenotyping of pooled gene deletion mutants using bar-seq and projection pursuit clustering revealed functional phenotypic groups of protein kinases involved in differentiation from metacyclic promastigote to amastigote, growth and survival in macrophages and mice, colonisation of the sand fly and motility. This unbiased interrogation of protein kinase function in *Leishmania* allowed targeted investigation of organelle-associated signalling pathways required for successful intracellular parasitism (Baker et al., 2021, Nat Comms 12:1244). We are now applying this approach genome-wide in the LeishGEM (http://leishgem.org/) collaborative project. We are using high-throughput reverse genetics to determine protein subcellular localisation of tagged proteins (~2,500) and function through fitness phenotyping of deletion mutants (~8,000). We are also using LOPIT-DC: Localisation of Organelle Proteins by Isotope Tagging after Differential ultraCentrifugation as an additional approach to determine protein location in Leishmania. These high throughput approaches will provide novel insights into the Leishmania-host interaction and will provide new therapeutic targets.

LeishGEM is funded by a Wellcome Trust Collaborative Award.