Leishmania braziliensis Protein arginine methyltransferases 1 and 3 are mutually dependent for activity

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Gene expression is carefully controlled in response to environmental stimuli in *Leishmania*. Post-translational modifications (PTMs) have important roles in regulating functions of proteins that carefully control gene expression. Arginine methylation is one such PTM, catalysed by protein arginine methyltransferases (PRMTs). We biochemically characterised PRMT homologs in *Leishmania braziliensis*: PRMT1, 3, 5, 6 and 7. We recombinantly expressed and purified each of these enzymes and assayed against substrates rich in arginine residues to identify intrinsic methyltransferase activity. *Lbr*PRMT5 and 7 demonstrate strongest activity on an RBP16$^{102\text{-}119}$ peptide and *Lbr*PRMT7 has broader substrate activity. K_m data measured confirm *Lbr*PRMT7 has highest affinity for the RBP16-derived peptide. *Lbr*PRMT6 demonstrated no activity at all against the substrates tested. Individually, *Lbr*PRMT1 and 3 were inactive against all the peptide substrates tested. Gel filtration data clearly show *Lbr*PRMT1:3 form a hetero-tetrameric complex in solution. This complex was confirmed and characterised via cryo-EM and incubating peptide substrates with *Lbr*PRMT1 and 3 together demonstrates strong methyltransferase activity. Methyltransferase assays with catalytically dead double E loop mutants of *Lbr*PRMT1 and 3 demonstrate *Lbr*PRMT1 is the active component of the complex. Finally, we assess activities of the *Lbr*PRMTs at different temperatures. We show that temperature affects each *Lbr*PRMT in a substrate-specific manner. Combined, our data indicate that *Lbr*PRMT1:3 has a very similar paradigm to *Tb*PRMT1^{ENZ}:1^{PRO}, however the retention of a complete double E loop in *Lbr*PRMT3 raises questions about the activity of the *Lbr*PRMT1:3 complex. Therefore, *Lbr*PRMT3 retaining the second glutamate of the double E loop may catalyse an alternative target or serve an unknown function. Moreover, our temperature assay data provides insight into the activities of *Lbr*PRMTs that suggests biologically-relevant, as yet uncharacterised layers of regulation.

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