

Identification of a BolA-like protein in *Giardia intestinalis*: a new Fe-S cluster assembly factor in mitomes

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The unifying feature of most mitochondria and related organelles (MROs) – including the metabolically reduced mitosomes of the metamonad *Giardia intestinalis* - is the compartmentalization of FeS cluster biosynthesis mediated by the iron-sulphur cluster (ISC) system. This assembly is generally divided into two sequential phases: the maturation of 2Fe-2S and 4Fe-4S-containing FeS cluster proteins respectively; the latter of which is poorly understood. It was recently shown that the BolA protein family plays a critical role in the maturation of 4Fe-4S iron-sulphur cluster proteins. Previous work has demonstrated that anaerobic organisms tend to encode more 4Fe-4S than 2Fe-2S proteins likely due to the higher oxygen-stability of the latter. To advance our knowledge of 4Fe-4S FeS cluster maturation in anaerobic protists, we explore the evolutionary history of BolA and its predicted partners in metamonads - a lineage comprised of free-living and parasitic anaerobic protists with MROs (*e.g.*, hydrogenosomes and mitosomes). We find that BolA is only found in *Giardia intestinalis*, *Dysnectes brevis* and *Kipferlia bilata* and not other metamonads. To test the subcellular localization of the *Giardia* BolA protein, we co-expressed a biotin acceptor peptide (BAP)-tagged BolA and BAP-specific biotin ligase (BirA) in *Giardia intestinalis*. Using immunofluorescence we find that BolA-BAP indeed localizes to the mitosome of *Giardia intestinalis*. We also present the interactome of the *Giardia* BolA protein using the BAP-BirA *in vivo* protein tagging method for analysing BolA-containing complexes. Although BolA has been implicated in the assembly 4Fe-4S proteins, we were unable to bioinformatically detect 4Fe-4S-containing proteins in the *Giardia* mitosome. This suggests that BolA might function in the maturation of a yet unknown 4Fe-4S-containing protein in the *Giardia* mitosome or perform an alternative role in FeS cluster assembly.