THE CHARACTERIZATION OF THE J-DOMAINS OF TRYPANOSOMAL TYPE-1 DNAJ-LIKE PROTEINS

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During their life-cycle, trypanosomes move from their poikilothermic invertebrate hosts to their homothermic mammalian hosts (37C body temperature). It is believed that trypanosomes utilize a heat shock protein system to acclimatize to the increased host temperature and thus facilitate the development of a trypanosomal infection. Two heat shock protein 40 (Hsp40) homologues have been the focus of this work: *Trypanosoma cruzi* J protein 2 (Tcj2) and *Trypanosoma cruzi* J protein 3 (Tcj3). Full length Tcj3 was unable to complement in a DnaJ minus *Escherichia coli* strain (OD259). Therefore, in order to study the J-domain of Tcj3, a chimera of the Tcj3 J-domain and the C-terminal side of the *Agrobacterium tumefaciens* DnaJ protein (Tcj3-J-Agt DnaJ) was generated. This chimera was able to complement in this system, suggesting a conservation of function of the J-domain. The mutation of histidine 33 to glutamine in the J domain of DnaJ in *Escherichia coli* has been shown to disrupt the function of the DnaJ/DnaK system. This mutation, along with mutations of highly conserved residues were introduced into Tcj2 and the Tcj3-J-Agt DnaJ chimera to investigate their effect on the function of the trypanosomal Hsp70/Hsp40 system *in vitro* and *in vivo*. ATP hydrolysis assays were performed on Tcj2 with its partner Hsp70 to test the effect of the mutations *in vitro*. The ability of the Tcj3-J-Agt DnaJ chimera mutants to complement in the *E. coli* OD259 system was assessed. Results from these experiments will be discussed.

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