ACTIVATION OF *Leishmania braziliensis* HSP90 BY AHA1 CO-CHAPERONE OCCURS THROUGH A COOPERATIVE MECHANISM

Seraphim, T.V.¹; Kakihara, Y.²; Houry, W.A.²; Borges, J.C.¹, #

¹Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, Brazil; ²Department of Biochemistry, University of Toronto, Toronto, Canada;  #borgesjc@iqsc.usp.br

INTRODUCTION: Hsp90 is a critical molecular chaperone involved in protein homeostasis, cell signaling, genome maintenance and transcriptional and translational processes. Hsp90 is a homodimer formed by three domains (N, M and C-domains) and undergoes several conformational changes during its functional cycle. Hsp90 has low ATPase activity and its functional cycle is driven by co-chaperones, such as Aha1. Aha1 is formed by two domains (N and C-terminal) and accelerates Hsp90 ATP hydrolysis through stabilization of Hsp90 in a closed state. **OBJECTIVES:** In spite of its importance for Hsp90 function, Aha1 proteins from protozoa are poorly studied and in this work we aimed to characterize the interaction mechanism between *Leishmania braziliensis* Aha1 (LbAha1) and Hsp90 (LbHsp90).

**MATERIAL AND METHODS:** *In vitro* interaction experiments between recombinant LbAha1 constructions (full length, N- and C-terminal domains) and LbHsp90 constructions (full length, NM- and M-domains) were performed by isothermal titration calorimetry and enzyme kinetics. Yeast W303a aha1Δ strains were generated to investigate the LbAha1-Hsp90 interaction *in vivo* by yeast complementation assays.

**RESULTS AND DISCUSSION:** LbAha1 N-terminal interacted with LbHsp90 M-domain via electrostatic and hydrogen bond interactions with stoichiometry of 2 LbAha1 per LbHsp90 dimer. The interaction of LbAha1 C-terminus with LbHsp90 was found to be LbHsp90 conformation-dependent, since a weak interaction was only seen when LbHsp90 was a dimer. Moreover, only the interaction of full length LbAha1 led LbHsp90 toward a closed conformational state. We found that only full length LbAha1 was able to stimulate the LbHsp90 ATPase activity by 10-fold. Yeast aha1Δ cells showed a temperature-sensitive phenotype and complementation assays are in progress to investigate the LbAha1 function *in vivo*. **CONCLUSIONS:** LbHsp90 ATPase activity stimulation involves both LbAha1 domains through a cooperative mechanism and only the full-length LbAha1 is functional *in vitro*.

**Keywords:** Molecular chaperones, Aha1, Hsp90, Leishmania.

**Financial support:** FAPESP, CNPq and CAPES.