On the interaction of the C-terminus of Human 90 kDa Heat Shock Protein Hsp90 with the Mitochondrial Translocase of outer Membrane Tom70

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INTRODUCTION. The mitochondrial import receptor Tom70 (translocase of the mitochondrial outer membrane 70) is a TPR-like protein, which interacts with chaperone–preprotein complexes and Hsp90 is a crucial molecular chaperone required for folding. Although it is known that the pentapeptide MEEVD at the C-terminus of Hsp90 is the motif involved in the recognition of TPR proteins, the molecular mechanism by which Tom70 co-ordinates interactions with preproteins and chaperones is still elusive.

MATERIALS AND METHODS. The interaction was investigated by cross-linking coupled with LC-MS/MS analysis, small-angle X-ray scattering (SAXS), isothermal tritration calorimetry (ITC) and in silico methods.

RESULTS AND DISCUSSION. Mass spectrometry (MS) analysis of the tryptic products of the complex revealed four interpeptide cross-link sites in the complex, from which two were sites between Tom70 and C-Hsp90. The interpeptide cross-link sites were confirmed by ITC experiments in which a model peptide of the region acted as a competitor for the complex interaction. Structural modeling based on cross-linking MS analysis was employed to unveil the molecular interfaces involved in the interaction and the complex model, which was supported by SAXS analysis.

CONCLUSION. This study constitutes the first direct mapping of interaction sites in the C-Hsp90/Tom70 complex and is a significant contribution to the mechanism of interaction between Hsp90 and co-chaperones.

Key words: Hsp90, Tom70, interface mapping, crosslinking, ITC, SAXS.

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