THE REGULATORY EFFECTS OF CELL CYCLE KINASES ON LOCALISATION AND FUNCTION OF THE MURINE STRESS INDUCIBLE PROTEIN 1 (mSTI1)

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The murine stress-inducible protein 1 (mSTI1) is a co-chaperone which mediates the formation of the Hsp70/mSTI1/Hsp90 chaperone heterocomplex. This chaperone machine plays an important role in the folding and regulation of various eukaryotic signaling proteins. mSTI1 is an *in vitro* substrate of cell cycle kinases which appears to regulate the subcellular localisation of this protein. Phosphorylation of mSTI1 by Casein kinase II (CKII) results in an increased nuclear accumulation of mSTI1, and phosphorylation by cell division cycle 2 kinase (cdc2 kinase) results in an increased cytoplasmic accumulation of mSTI1. These phosphorylation sites are found just upstream of the central tetratricopeptide repeat domain (TPR2A), through which mSTI1 interacts with Hsp90. Moreover, a putative nuclear localization signal (NLS) sequence has been identified that overlaps with the TPR2A domain. It is thus speculated that Hsp90 may be involved in regulation of the nuclear localization of mSTI1. Site-directed mutagenesis and surface plasmon resonance (SPR) spectroscopy were used to determine whether phosphorylation of mSTI1 by the above mentioned kinases affect its interaction with Hsp90. The results demonstrated a diminished affinity for interactions between the cdc2-phosphorylated mimic of mSTI1 and Hsp90 (KD value of 2.5 uM, whereas the CKII-phosphorylated mimic of mSTI1 did not show a significant deviation in affinity for Hsp90 (KD: 1 uM) from full-length mSTI1 (KD: 1.4 uM). Interestingly, a triple mutation of the second arm of the proposed bipartite NLS (K237/238/239A) completely abolished interactions of mSTI1 with Hsp90, whereas, a single mutation within this region (K239A) reduced the level of mSTI1-Hsp90 interactions by about 70

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