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D.71- Obtaining a recombinant GFP fused to a C-terminal degron for interacting test with human Hsp70s

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70 kDa heat shock proteins of (Hsp70 or HSPA in humans) are involved in protein quality control, also participating in the identification and routing of misfolded proteins for degradation via the ubiquitin-proteasome system through a complex mechanism involving the cochaperone CHIP (C-terminus Hsp70/Hsp90 interacting protein), a ubiquitin ligase. Using a cellular model containing an eGFPuv variant with a degron sequence at the C-terminal (eGFPuv-dCL1), we observed that inhibition of human Hsp70 results in the accumulation of GFP. This variant would normally be degraded via proteasome, which when inhibited via MG-132 also causes GFP accumulation. These results suggest the interdependence of Hsp70 and the eGFPuv-dCL1 depuration. The objective of this study is to produce the recombinant protein eGFPuv-dCL1 from the vector pET28a and characterize its secondary and tertiary structure. Subsequently, to investigate the physical interaction of eGFPuv-dCL1 with recombinant human Hsp70. The expression of the eGFPuv-dCL1 protein was performed in Escherichia coli BL21(DE3) strain. Purification of eGFPuv-dCL1 was carried out in two chromatographic steps: nickel affinity chromatography and size exclusion chromatography. Purity was estimated by 12% SDS-PAGE. The secondary structure of eGFPuv-dCL1 was analyzed by circular dichroism, and the tertiary structure of eGFPuv-dCL1 was evaluated by fluorescence emission at ~490 nm. Cellular studies demonstrate that inhibition of human Hsp70 by the MKT-077 inhibitor causes the accumulation of eGFPuv-dCL1 in cells, suggesting a failure in delivering this client protein to the ubiquitin-proteasome system due to overload of the chaperone system. The recombinant eGFPuv-dCL1 protein was properly purified, with 95% purity, and its secondary and tertiary structural content indicate that it was produced folded. With this protein, interaction studies with human Hsp70 with be performed using a full-length recombinant eGFPuv as a control. With the pure and properly folded recombinant protein eGFPuv-dCL1, it will be possible to test its interaction with human Hsp70 and reproduce the observed cellular results. Keywords: eGFPuv-dCL1, Hsp70, Molecular chaperone

D.72- Ligand screening using thermal shift assays of the periplasmatic domain of ABC transport Rv2563/Rv2564 of Mycobacterium tuberculosis

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In the infection and survival processes of Mycobacterium tuberculosis (Mtb) after entering the macrophage, it is notable its capacity to remodel the cell envelope, escape from the immune system and upregulate genes that increase the resistance to antimicrobial agents. Among many proteins, ATP-Binding Cassette (ABC) transporters are highly prominent in these processes.Within these transport systems, the ABC transporter Rv2563/Rv2564 stands out. Its function and the three-dimensional structure are not fully understood, but proteomic analysis has related its production under treatment of the bacillus with different drugs. test if Rv2563-per was capable to interact with different ligands or suffer structural changes induced by pH To test if Rv2563-per was capable to interact with different ligands or suffer structural changes induced by pH, the domain was expressed in E. coli (BL21) cells and purified by immobilized metal affinity chromatography and size exclusion chromatography. The purified domain was submitted to thermal shift assays (TSA), in a set of buffers with different pH (4 to 11) and presence of amino acids L-glutamine, L-asparagine, and glutamic acid. The results showed that the amino acids did not interfere in the thermal stability of the domain, but acidic pH, specifically 4.0-5.0. To complement these results, the secondary structure content of Rv2563-per was evaluated in the same conditions by circular dichroism. The results revealed a gain of secondary structures under acidic conditions. These results might suggest that the transporter suffers structural changes inside the macrophage, during the acidification process. Keywords: thermal shift, ligand, tuberculosis