

P033 A possible role for Hsp90 in the prenylation of Rab GTPases
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Rab GTPases are key regulators of eukaryotic membrane trafficking. Rabs require lipid modification, namely geranylgeranylation for membrane association and function. Geranylgeranylation of Rab proteins is catalysed by Rab geranylgeranyl transferase (RGGT) and assisted by Rab Escort Protein (REP). REP associates with newly synthesised unprenylated Rabs, presents them to RGGTase then escorts the prenylated Rab proteins to their target cellular membranes. To further understand the role of REP in Rab geranylgeranylation, we attempted to find REP-interacting proteins. Immunoprecipitation of REP from rat and bovine brain cytosols led to the identification of three proteins, one of which was identified as Hsp90 by microsequencing. *In vitro* prenylation assays showed that addition of recombinant Hsp90 enhanced the REP/RGGTase-mediated prenylation of several Rab proteins. Addition of the Hsp90 inhibitor geldanamycin to HeLa cells shortly after transfection with EGFP-Rab5a had no effect on the partition of the newly expressed Rab protein into the detergent phase following Triton-X114 extraction or localisation to early endosomes in fixed cells. Furthermore, treatment with mevastatin (an inhibitor of HMG CoA reductase) but not geldanamycin resulted in the accumulation of endogenous unprenylated Rabs in the cytosol of HeLa and HEK293 cells. S³⁵-methionine pulse-chase experiments showed that inhibition of Hsp90 had no effect on the half-life of hREP1. We conclude that Hsp90 appears to associate with REP in cytosol and to promote REP activity *in vitro*. However, the role of Hsp90 in the function of REP *in vivo* remains unclear.