

P020 The function of HD-PTP in endosomal sorting and morphogenesis

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Downregulation of mitogenic receptors involves their internalisation and sorting into intraluminal vesicles of multivesicular bodies (MVBs). Receptors are ultimately degraded in the lumen of lysosomes. Several evolutionary conserved complexes (Endosomal Sorting Complexes Required for Transport; ESCRTs) and accessory proteins play a role in MVBs function. The yeast protein Bro1p binds ESCRT-III via its N-terminal Bro1 domain and recruits the deubiquitinating enzyme Doa4p. Its mammalian homologue has yet to be clearly identified. We have set out to investigate the function of the mammalian Bro1p-like protein His domain protein tyrosine phosphatase (HD-PTP). Depletion of HD-PTP by RNAi caused an accumulation of EGF receptor and ubiquitinated proteins in aberrant endosomal compartments. This phenotype could be rescued by expressing an RNAi-resistant form of HD-PTP. To assess the functional relevance of the domains of HD-PTP, rescue experiments were performed using either point mutations or deletion mutants affecting the Bro1-, V- and proline rich- domains. Endosomal recruitment of the mutants was assessed by immunofluorescence microscopy upon co-expressing them with a dominant negative form of the AAA ATPase, VPS4. The Bro-1 domain is essential for the endosomal targeting of HD-PTP, while the V-domain is crucial for its function. The ESCRT-I binding PR-domain is dispensable both for HD-PTP localisation and function.