

NHE8 is a negative regulator of protein sorting at the multivesicular body

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Studies in yeast have identified 18 proteins (the class E Vps proteins) required for the sorting of proteins into the luminal membranes of multivesicular endosomes. Of these, 17 are soluble or peripherally membrane-associated proteins. The only transmembrane class E Vps protein is the sodium/ proton exchanger Nhx1p. Putative orthologues of the soluble class E Vps proteins have been identified, and some of these orthologues have been shown to play similar roles to their yeast counterparts in multivesicular body sorting. We have focussed on identifying the mammalian orthologue of Nhx1p. Nhx1p belongs to the NHE family of sodium/ proton exchangers, and falls into the intracellular subfamily of these exchangers based on protein sequence similarities. Based on this analysis, the candidate orthologues of Nhx1p are NHE6, 7, 8 or 9. We have studied the function of NHEs 6, 7, 8 and 9 in multivesicular body protein sorting using the degradation of epidermal growth factor (EGF) as a functional assay. Depletion of NHE8 (but not NHE6, 7 or 9) using siRNA results in an increase in EGF degradation. This phenotype is similar to that seen on depletion of the ESCRT III-associated protein Alix. In addition, NHE8-depleted cells have altered endosomal morphology by immunofluorescence, and an increase in intraluminal vesicles within multivesicular bodies by electron microscopy. We are currently studying the role of NHE8 in endosomal protein trafficking in further detail, but propose that NHE8 is the mammalian orthologue of yeast Nhx1p.