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# Role of CHMP5 in Endosomal Trafficking

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# Role of CHMP5 in Endosomal Trafficking

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The Endosomal Sorting Complex Required for Transport (ESCRT) machinery is a set of proteins that help with the formation of intraluminal vesicles (ILVs) inside of endosomes. A complex of this machinery, ESCRT-III proteins, are thought to drive the membrane remodeling and fission required for this process. One ESCRT-III protein, CHMP5, is essential for life but has been shown to not be required for ILV formation, so its specific role in endosomal trafficking is unclear. In this study, we used siRNA to transiently deplete CHMP5 in cultured human cells. We found that CHMP5 depleted cells had an accumulation of enlarged swollen particles (diameters greater than 480nm) that were positive for late endosomal marker LAMP1 and an increase in the number of small LAMP1 endosomes (diameters less than 300nm). In CHMP5 depleted cells, large endosomes were also shown to uptake FM dye and stain positively for CD63, suggesting that they are full of ILVs—which agrees with previous reports. We thought that this increase in ILVs might be due to impaired lysosome formation or degradative capacity. We found that Magic Red, a marker for degradative capacity, was present, and even increased, in CHMP5 depleted cells, indicating that lysosome formation was not impaired. The large swollen endosomes were MR negative which suggested that they are likely late endosomes. Given that lysosome formation was not impaired, we then looked at the maturation of late endosomes. This process involves a well-characterized recruitment of retromer, a complex that removes early endosomal membrane cargo from the maturing endosome. We found that SNX1 and VPS35, the co-localization of which is required for a functional retromer complex, were less co-localized in CHMP5 depleted cells. All together, these data suggests that CHMP5 depletion does not affect lysosome function, but may impair the maturation and fusion of late endosomes with lysosomes.