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MATERIALS AND METHODS

Cloning

DNA encoding Vps4 from *S. cerevisiae* was amplified by PCR from yeast genomic DNA, and directionally cloned into the pET151 *E. coli* expression vector (Invitrogen). Constructs expressing mutant proteins were created by Quick-change mutagenesis (Stratagene) and verified by DNA sequencing.

Protein Expression and Purification

$Vps4_{122\text{-}437,E233Q}$ and $Vps4_{104\text{-}437,E233Q}$ were expressed in BL21 Codon+ *E.coli* cells (Stratagene) in ZY autoinduction media for 6 h at 37°C and then overnight at 21°C. Cells were harvested by centrifugation and lysed by resuspension in 10 mg/ml lysozyme in lysis buffer (10 mM imidazole, 300 mM NaCl, 50 mM Tris pH 7.4, 5% glycerol and protease inhibitors (PMSF, aprotinin, leupeptin, pepstatin), 45 min, 4°C), followed by sonication. The lysate was clarified by centrifugation (45 min, 35 000 x *g*) and the soluble Vps4 proteins were bound to a Ni^{2+} sepharose column (Amersham), washed with 10 column volumes of lysis buffer, and eluted in 75 mM imidazole (in lysis buffer). Fractions were assayed for Vps4 by SDS-PAGE, pooled, dialyzed into 100 mM NaCl, 25 mM Tris pH 7.4, and 1 mM DTT, and purified by anion-exchange chromatography (Q-sepharose, Invitrogen, 100-500 mM NaCl gradient in 25 mM Tris pH 7.4, and 1 mM DTT). Vps4 fractions were pooled, and the $(His)_6$ affinity tag was removed by incubation with TEV protease (~1 mg/100mg protein, 20 h at 4°C), followed by dialysis into 20 mM Tris pH 7.4, 100 mM NaCl, and removal of unprocessed protein by Ni^{2+} sepharose chromatography. The cleaved protein was