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Towards the Cryo-EM structure of the ERAD glycoprotein folding checkpoint, the EDEM:PDI heterodimer.

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The 130 kDa ER Degradation Enhancing Mannosidase (EDEM) selectively de-mannosylates an N-linked glycan on terminally misfolded glycoproteins, thereby dispatching it to retrotranslocation, cytosolic ubiquitination, and proteasome degradation. EDEM activity depends on association with a 60 kDa Protein Disulphide Isomerase (PDI) to form the EDEM:PDI heterodimer [1-3]. No EDEM selective inhibitors are known, and yet EDEM modulators would have therapeutic potential in virology, rare genetic disease and cancer, as well as constituting important reagents for biotechnology and agricultural science. No EDEM structures have been published yet. Furthermore, the molecular determinants of misfold recognition and selective de-mannosylation of the C-branch of the substrate N-linked glycan by EDEM:PDI remain elusive to date. We selected the Chaetomium thermophilum EDEM and PDI (CtEDEM and CtPDI, respectively) and confirmed that CtEDEM is an EDEM in a plant model. CtEDEM and CtPDI (the latter without its ER retrieval signal) were cloned in the pHLsec vector for secreted expression in mammalian cells. Four days after co-transfection of HEK293F cells, the CtEDEM:CtPDI is purified from the cells supernatant by IMAC followed by SEC. The purified complex was vitrified on a transmission cryo-electron microscopy (Cryo-EM) grid. Data collection yielded a total of 11208 micrographs from which a total of 1,850,000 particles were picked and selected by rounds of 2D classification. A subset of 180,000 particles gave a 3.5 Å reconstruction of the complex, with approximate dimensions 120x90x70 Å. Phased molecular replacement has been used to dock domains from models of structurally similar proteins from the AlphaFold 2.0 database. The EDEM catalytic domain nestles inside the curved arc formed by the four thioredoxin domains of the PDI. The two topologically intertwined C-terminal EDEM domains [4] stick out of the main body of the complex. Each of two EDEM free Cys residues are within disulphide bonding distance from one of the two redox-active SS bonds of the PDI, suggesting that redox chemistry is important for the function of the enzyme. Structure-based hypotheses about the function of EDEM:PDI ERAD and experiments to test them will be discussed.

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