Oral Contributions

[MS6-04] Structural Studies of Proteins Involved in ER-associated Protein Degradation. Udo Heinemann, Anup Arumughan, Jennifer Hanna, Yvette Roske, Anja Schütz, Erich E. Wanker

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Proteins are translocated into the endoplasmic reticulum of cells in an unfolded state and acquire their native conformation in the ER lumen after signal-peptide cleavage. ER-associated degradation (ERAD) of folding-incompetent protein chains is mediated by protein complexes residing in the ER membrane. We study the architecture and function of one of these, the HRD complex assembled around the E3 ubiquitin ligase Hrd1.
The recognition of ERAD substrates is linked to the maturation of their carbohydrate structures. The HRD complex-associated lectin Yos9 is involved in ERAD substrate recognition by binding carbo-hydrates through its mannose-6-phosphate receptor homology (MRH) domain. We have determined the crystal structure of a central domain of Yos9, adjacent to the MRH domain, which was previously annotated as interaction region with the HRD subunit Hrd3 [1]. We find that this domain does not support Hrd3 association which we map to the N-terminal half of Yos9 instead. In contrast, the domain has a function in Yos9 dimerization as seen in the crystal structure, in various solution experiments and as supported by mutagenesis of dimer interface residues. The dimerization of the ER-luminal Yos9, in conjunction with studies of the cytosolic domain of the HRD component Usa1 [2] and other biochemical data thus supports a model of a HRD complex that exists and functions as a dimer or higher multimer.
The delivery of ubiquitinated ERAD substrates to the proteasome is mediated by the cytosolic AAA+ ATPase Cdc48 (p97 in mammalian cells). AAA+ enzymes are ATPases Associated with diverse cellular Activities. p97 (VCP) serves a wide variety of cellular functions in addition to its role in ERAD, including organelle membrane fusion, mitosis, DNA repair and apoptosis. These different functions are linked to the binding of adaptor proteins to p97 many of which contain ubiquitin regulatory X (UBX) domains. As shown by crystallographic analysis, one of these adaptors, ASPL (alveolar soft part sarcoma locus), uses a substantially extended UBX (ubiquitin regulatory-X) domain for binding to the N domain of p97 where a lariat-like, mostly α-helical extension wraps around the N-terminal domain of one subunit of p97. By this binding ASPL triggers the dissociation of functional p97 hexamers and the formation of p97-ASPL heterotetramers, leading to partial inactivation of the AAA+ ATPase. To the best of our knowledge, this is the first time that the structural basis for adaptor protein-induced inactivation by hexamer dissociation of p97 and, indeed, any AAA+ ATPase has been demonstrated. This observation has far reaching implications for AAA+ ATPase-regulated processes.
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[1] Hanna, J., Schütz, A., Zimmermann, F., Behlke, J., Sommer, T. & Heinemann, U. (2012). Structural and biochemical basis of Yos9 protein dimerization and possible contribution to self-association of 3-hydroxy-3-methylglutaryl-coenzyme A reductase degradation ubiquitin-ligase complex. *J. Biol. Chem.* **287**, 8633-8640.
[2] Horn, S.C., Hanna, J., Hirsch, C., Volkwein, C., Schütz, A., Heinemann, U., Sommer, T. & Jarosch, E. (2009). Usa1 functions as a scaffold of the HRD-ubiquitin ligase. *Mol. Cell* **36**, 782-793.

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