Summary PhD Thesis - Christian Schuberth

The abundant, highly conserved AAA ATPase Cdc48 from yeast (p97 in mammals) is involved in various cellular processes including homotypic membrane fusion and ubiquitin-dependent protein degradation. The underlying mechanism of Cdc48 action is believed to be the ATP-dependent disassembly of oligomeric protein complexes. The specificity of this "segregase" activity is determined by distinct, mutually exclusive cofactors, which recruit the hexameric Cdc48 complex to it's frequently ubiquitylated substrates. The heterodimeric adaptor Ufd1-Npl4 is required mainly for functions in the ubiquitin/proteasome pathway, whereas the cofactor Shp1 (p47) has been linked to membrane fusion processes. Shp1 harbours at its C-terminus a "ubiquitin regulatory X" (UBX) domain, a domain with low sequence homology but high structural similarity to ubiquitin. The UBX domain has been identified in a variety of eukaryotic proteins from yeast to man, defining a new and largely uncharacterized protein family. The aim of this study was to investigate the cellular functions of UBX domain proteins in yeast.

All seven UBX proteins of *Saccharomyces cerevisiae* (Shp1 and Ubx2 to Ubx7) were found to interact with Cdc48 and the UBX domain was identified as the key binding determinant. A subgroup of UBX proteins containing a ubiquitin associated (UBA) domain is able to bind to ubiquitin and ubiquitylated proteins. Two of these UBA/UBX proteins, Shp1 and Ubx2, were shown to be involved in the degradation of a model substrate of the ubiquitin/proteasome system. Furthermore, Ubx2 was identified as a new component of the ERAD (ER-associated degradation) pathway, a quality control system to remove misfolded proteins from the endoplasmic reticulum (ER). Before they are degraded by the proteasome, ERAD substrates need to be extracted from the ER via a dislocation pore, a process involving $Cdc48^{Ufd1\text{-}Npl4}$. In contrast to Shp1, Ubx2 and Ufd1-Npl4 binding to Cdc48 was found to be not mutually exclusive, but to occur simultaneously, resulting in a stable $Cdc48^{Ufd1\text{-}Npl4\text{-}Ubx2}$ complex. Ubx2 is an integral ER membrane protein and recruits $Cdc48^{Ufd1\text{-}Npl4}$ to ERAD substrates, E3 ubiquitin ligases and putative dislocation pore components, thus connecting the ubiquitylation and translocation activities required for efficient ERAD.

Taken together, UBX domain proteins represent a new family of Cdc48 cofactors, providing the potential to assemble a large number of different Cdc48 adaptor complexes to specify the various activities.