Summary

Protein degradation is necessary to maintain normal cellular homeostasis. Many human diseases are caused through the accumulation of unfolded or misfolded proteins in the ER. These proteins are recognized by the quality control system, which protects the ER from overload. In the first step, components of the quality control system try to refold improperly folded polypeptides to achieve their native and transport-competent conformation. If the amount of misfolded proteins exceeds the capacity of the folding machinery, their accumulation can activate the unfolded protein response. This cellular stress response pathway attenuates translation, up-regulates the chaperones required for proper protein folding and activates the ER-associated degradation (ERAD) pathway. Subsequently, terminally misfolded proteins are translocated to the cytosol and are rapidly degraded by the ubiquitin / proteasome system. The 26S proteasome is a new important drug target and the inhibition of its catalytic activities plays a pivotal role in the treatment of multiple myeloma, a cancer of bone marrow cells. Since general proteasomal inhibition is accompanied by severe side effects, the modulation of ERAD may be an interesting approach in the treatment of folding diseases like nephrogenic diabetes insipidus or cystic fibrosis.

The V2 vasopressin receptor (V2R) belongs to the family of G protein-coupled receptors (GPCR), which regulate essential signal transduction pathways including the V2R-mediated vasopressin-activated water reabsorption in the collecting duct of the kidney. GPCRs also constitute important drug targets for a multitude of diseases. More then 170 different mutations of the gene encoding for the V2R are described to cause nephrogenic diabetes insipidus, a severe disorder characterized by polyuria and polydipsia. Most of them are point mutations that cause substitutions of single amino acids. The mutations are evenly spread over all the domains of the receptor and may lead to V2R mutants that are retained in several compartments of the secretory pathway by the quality control system. The mutants included in this study were localized in the ER, in the ER-Golgi intermediate compartment, and in the Golgi apparatus. This thesis aimed to characterize their degradation pathways.

The findings presented here demonstrate that the quality control system determines the degradation pathway of V2Rs. Wild-type and mutant receptors with folding defects are recognized by the quality control system of ER (wild-type, L62P), ERGIC (V226E) and Golgi apparatus (G201D). Misfolded receptors that are able to escape the quality control of the ER and ERGIC traffic to the Golgi apparatus, acquire the mature glycosylation state, and are degraded in lysosomes. ERGIC-retained mutants are most likely trans-

ported back to the ER and all misfolded, immature ER-resident receptor forms are subsequently eliminated via the ERAD. The human ERAD is not well characterized; the molecular mechanisms are still unclear and there are many open questions concerning the function and organization of the precise dissection of the events including participation of unique ERAD components.

The data presented in this study reveal that the ERAD is involved in turnover of all ER-retained, immature receptor forms (wild-type and mutant receptors). The V2Rs co-precipitate with a canonical ERAD component, the AAA ATPase p97/valosin-containing protein (p97/VCP). p97/VCP participates in all known ERAD pathways and is presumably involved in the extraction of proteins out of the ER membrane. During retrotranslocation, the V2Rs are polyubiquitinated by an unknown ubiquitin ligase. Polyubiquitination enhances the interactions between components of the retrotranslocation machinery. V2Rs with mutations in different receptor domains (L62P, cytosolic; V226E, transmembrane; G201, ER-luminal) can be co-precipitated with the potential channel protein Derlin-1. This is in striking contrast to recently published results obtained in yeast, where three distinct ERAD pathways operate. The activation of these pathways depends on the localization of the defect in the protein structure: ERAD-M (membrane), ERAD-L (luminal) or ERAD-C (cytosolic). The findings presented here demonstrate that only one p97/VCP / Derlin-1-dependent ERAD pathway degrades receptors bearing different misfolded domains in a mammalian system. In subcellular fractionation experiments, proteins from cytosolic and membrane compartments were separated by centrifugation. This study revealed that deubiquitinated and deglycosylated V2Rs are released into the cytosol prior to degradation.

In search of new interaction partners, co-precipitated proteins of wild-type V2Rs were identified in mass spectrometry experiments in the presence of proteasome inhibitors. It could be demonstrated that Rpt1, a regulatory subunit of the 19S particle of the 26S proteasome and a component of a second ATPase was pulled down with the wild-type V2R. Until now, it is not fully understood which subunit of the 19S complex facilitates the recognition of polyubiquitinated substrates targeted for degradation by the 26S proteasome. Rpn10, a non-ATPase subunit of the regulatory particle, could not be detected in V2R samples. Both Rpt1 and Rpn10 are described to bind polyubiquitin chains. One may hypothesize that the Rpt1 subunit facilitates recognition and binding of polyubiquitinated V2 receptors and leads to their proteasomal degradation.

In this thesis, a novel model system to understand the molecular functions of the human ERAD was established. The multiplicity of disease-causing mutations in all domains of the V2R provides an interesting tool to investigate the connection between the ERAD pathway and the ER / post-ER quality control function in a mammalian system.