Summary

The homeostatic equilibrium in the eukaryotic cells is achieved by permanent destruction and rebuilding of the cellular components. The regulation of these processes is essential for the appropriate response to genetic or environmental changes. Besides the synthesis and the destruction of highly abundant household proteins, the quantitative control of the less abundant regulatory proteins are very important for the process of the adaptation, development and reproduction. The controlled proteolysis of these regulatory proteins is one of the essential functions of the 26S proteasome. In addition, elimination of misfolded or damaged proteins arising as a consequence of different stresses is an other and very important function of the 26S proteasome.

This thesis summarizes our results on the structural properties of 26S proteasome, one of the main components of the ubiquitin - 26S proteasome pathway responsible for the regulated protein degradation.

In this pathway, an enzyme cascade, the ubiquitin ligase system selects and labels the short-lived or misfolded proteins by the covalent attachment of a polypeptide chain, called ubiquitin. The first two components of this system are mainly involved in the activation and transfer of the ubiuqitin, whereas the more diverse third components perform the selection of substrates for labelling. This selection is based on the primary degradation signals present in the proteins in the form of a short recognition sequence or a peculiar structure. The labelling process continues following the insertion of the first ubiquitin, but a multiubiquitin chain is being formed by addition of subsequent ubiquitin units. Certain types of the poliubiquitin synthesis reactions may require the fourth component of the ligase system.

The labelled proteins are degraded by a multiprotein complex, called the 26S proteasome, to small oligopeptides and reusable ubiquitin monomers. This multiprotein complex consists of two different subcomlpexes. One of them is the centrally positioned 20S proteasome. This tube-like structure has a narrow central channel, which connects three cave-like flares. The catalytic centers of this multicatalytic protease are located in the central cavity.

The compartmentalization of the 20S proteasome is essential to protect cellular proteins not intend for degradation.

The second subcomplex of the 26S proteasome, the 19S regulatory complex is connected to one or both ends of the barrel-shaped 20S proteasome. The selection of the ubiquitinated proteins and their feeding into the central channel of the 20S proteasome is the most important function of the 19S regulator complex. To perform this complex function the regulatory complex has to recognise and bind the ubiquitinated substrate proteins, its reverse chaperon activity unfolds the proteins, and opens the gated central chanel of the 20S proteasome.

The cleavage of the peptide bonds is not ATP-dependent, but both the substrate selection and its feeding into the 20S proteasome are accompanied by ATP hydrolysis. This is the cost of the high selectivity.

We have plenty of information about the enzymatic functions of the 20S proteasome. In addition, its structure analysed by X-ray crystallography is well-known. Unfortunately, our knowledge on the structure and function of the regulatory complex is far less advanced. Its subunit composition dynamically changes during the development and the environmental adaptation, producing a diverse population. The exact role of the particular subunits remains to be elucidated.

Our first aim was to create the complete subunit catalogue of the 26S proteasome. To address this issue we used a novel 16-BAC/SDS gel electrophoretic system used previously for the separation of highly hydrophobic integrant membrane proteins. For the analysis of the conformational changes accompanying the assembly of the 26S proteasome subunit-specific antibodies were assigned to all the subunits. During the annotation of the antibody library and analysing our cross-linking experiments several observations suggested that certain subunits of the regulatory complex may be present in posttranslationally modified forms. This observation led us to investigate in detail the possible O-linked N-acetylglucosamin modifications on the subunits of the 26S proteasome.

We have separated all the subunits of the 26S proteasome and identified two novel subunits. Mass spectrometric identification of the resolved subunits cut out from the gels was performed by our collaborators. In this way, we have established a complete subunit catalogue

of the *Drosophila* 26S proteasome. Furthermore, a regulatory complex subunit with deubiquitinating activity was identified, its enzymatic behaviour was characterised and the precise location of this subunit within the 26S proteasome particle have been mapped.

The assignment of the subunit specific antibodies to the subunits was performed as follows. Having separated the subunits of the 19S regulatory complex on identically prepared 16BAC/SDS 2D gels, we compared the stained gel pattern with the immunoblot patterns developed with different antibodies. After characterization of the recognition specificities, the antibodieswere ready to use for cross-linking studies.

In the cross-linking experiments using bifunctional protein cros-linkers, the closely spaced subunits within a multiprotein complex can be covalently cross-linked, resulting in heterodimers, recognised by both subunit-specific antibodies. With this technique we could examine the changes of the spatial distribution of the subunits following the ATP-dependent assembly or diassembly of the 26S proteasome. Our experiments revealed that the ATP dependent assembly of the 26S proteasome from 19S regulatory complex and 20S proteasome is accompanied by gross structural rearrangement of the constituent subcomplexes. We also demonstrated that certain regions of the subcomplexes are more involved in this structural rearrengement than others.

In the third set of experiments the presence of a specific postsynthetic modification was analysed in the subunits of the proteasome. O-linked N-acetylglucosamine modifications were detected on 2D gel separated subunits of the proteasome by immunoblot and lectin-binding assays. In the immunoblot experiments monoclonal antibodies, specific for O-linked N-acetylglucosamine modification were used, while the specificity of the wheat germ agglutinin for O-linked N-acetylglucosamine modification was exploited in the lectin-binding experiments. The high specificity of these reagents for this specific posttranslational modification has been well documented in the literature. We demonstrated that five subunits of the 19S regulatory complex and almost all the subunits of the 20S proteasome are N-acetylglucosamine modified. The physiological role of this extensive modification is still unknown. However, since it is known that the same serine and threonine residues can be phosphorylated or modified by the N-acetylglucosamine, we hypothetise that these two antagonistic modifications are able to fine-tune the function of the 26S proteasome.