Structure and Function Analysis of the Protein Degradation Machinery

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Scientific background

The proteasome is a 2.5 MDa molecular machine which catalyzes protein degradation. It is the central enzyme of the ubiquitin pathway of protein degradation. This pathway is central to an incredible multitude of processes and is involved in a wide range of biological aspects, including degradation of improperly or unfolded proteins, activation of the immune system, regulation of cell cycle progression, receptor-mediated endocytosis and signal transduction (Coux et al, 1996; Gonzalez et al, 2002; Pickart & Cohen, 2004; Szutorisz et al, 2006; Voges et al, 1999).

The 26S proteasome is composed of two multimeric subcomplexes: the proteolytically-active central 20S core particle (CP) and the 19S regulatory particle (RP). The core particle is constituted of 28 subunits and forms a cylindrical stack of four heptameric rings (~700 kDa), whose proteolytically active sites are sequestered within an internal chamber (Groll et al, 1997; Thrower et al, 2000). The regulatory particle (~900 kDa), can be further dissociated into two subcomplexes: 9 subunits compose the lid, and the remaining 10 subunits are part of the base. The former contains six ATPases and links the RP to the CP (Braun et al, 1999). The addition of RP to either one or both ends of the 20S creates the 26S proteasome. The 19S RP is involved in recognition of poly-ubiquitinated proteins (Lam et al, 2002; Strickland et al, 2000), deubiquitination, unfolding and translocation of the substrates into the CP (Braun et al, 1999; Reed & Gillette, 2007).

Overall, the ubiquitin-proteasome degradation pathway is crucial for maintaining cellular homeostasis. Misregulation of this process may lead to cancer and different neurodegenerative diseases. Our group aims to understand the structure-function relationships of the ubiquitin-proteasome proteolytic pathway. In particular, our research focuses on the following issues:

1. The 19S RP is implicated in recognition, unfolding and translocation of degradation substrates into the 20S CP. The precise arrangement of subunits within the 19S complex and its interface with the 20S remain unclear. Using different biochemical approaches coupled with mass spectrometry we are working towards structural characterization of this dynamic nanomachine.

2. "Degradation by default" is a recently discovered pathway which involves degradation of intrinsically unstructured proteins via the 20S CP and bypasses the traditional 19S-mediated degradation (Asher et al, 2006; Asher & Shaul, 2006; Orlowski & Wilk, 2003; Shringarpure et al, 2001). We aim to further study this unique process and reveal its regulatory mechanism.

3. ER Associated Protein Degradation (ERAD) is a mechanism by which misfolded proteins from the ER are degraded by the 26S proteasome. We are investigating the pathway by which aberrant proteins are targeted back to the cytosol and access the proteasome for degradation.

4. The COP9 signalosome (CSN) is a multisubunit complex composed of up to eight distinct subunits and is highly homologous to the lid sub-complex of the 26S proteasome (Chamovitz et al, 1996; Wei et al, 1994; Wei et al, 1998). Recent evidence from several systems indicate the existence of smaller sub-complexes ('mini'-CSN), which may have different activities than the holo-CSN complex (Mundt et al, 2002; Oron et al, 2002; Tomoda et al, 2002). We are interested to study the composition of mini-CSN sub-complexes and determine their functional relevance.

To address the above mentioned questions our laboratory uses a novel mass spectrometry approach for the analysis of multi-protein complexes at their native state.

Many aspects of the formation and composition of multi-protein complexes are difficult to define since assemblies may form only transiently or exhibit a dynamic subunit composition, and their function may change accordingly. Assembly may be further controlled *in vivo* by temporal and spatial coordination of protein expression and post-translational modifications. To understand how a macromolecular structure translates into function it is necessary to determine the characteristics of the complex and the contribution of each subunit to the overall activity (figure 1).

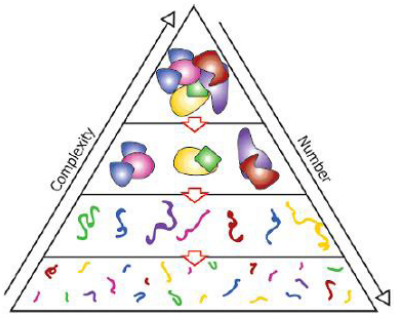

Fig. 1 *Pyramid of protein organization states. Various levels of protein organization can be probed in proteomic analyses by means of MS. A protein complex may be comprised of several subcomplexes, which themselves are composed of individual protein chains. A further level, that of peptide segments which are generated by proteolytic digestion, is the focus of traditional proteomics. Combining investigations into all four levels of organization however can allow for a far more extensive and detailed characterization of proteins and their functional states (Benesch et al, 2007).*

Recent developments in mass spectrometry (MS) have demonstrated that it is possible to provide key information about molecular assemblies, which are often too

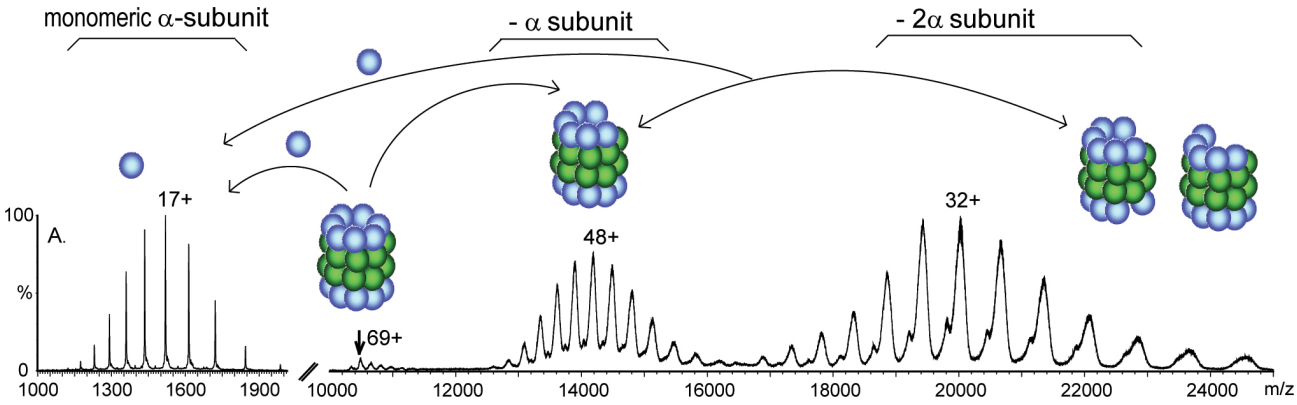

Fig. 2 *Tandem mass spectrometry (MS/MS) spectrum of the 28-mer (α14:β14) 20S proteasome from Rhodococcus erythropolis. Dissociation of the complex generates product ions from the loss of only the exposed α-subunits, consistent with the known architecture of the intact complex. Peaks between 12,000 and 16,000 m/z correspond to the loss of one α-subunit, and the series at 17,000–25,000 m/z corresponds to the loss of two α-subunits. At low m/z, 1000–2000 series of peaks are assigned to individual α-subunits (Sharon et al, 2007).*

large, heterogeneous or flexible to be amenable by established structural biology methods. The fundamental advantage of the MS approach lies in its ability to probe transient, asymmetric and heterogenous macromolecular complexes, using very low concentration of sample (figure 2). Insight into subunit stoichiometry, composition and structural arrangements can be revealed. In addition, the speed of the analysis enables dynamic reactions to be monitored in real time (Benesch et al, 2007; Sharon & Robinson, 2007; van den Heuvel & Heck, 2004).

Research objectives

Function and Structure of the 19S

The 19S regulates the activity and specificity of protein degradation (Braun et al, 1999; Rubin et al, 1998). It is composed of 19 different subunits which are arranged in two substructures, the lid and base. Characterization of the structure and function of the 19S is extremely difficult because of its complexity and dynamic nature. Our efforts are directed towards forming a comprehensive interaction map of each and every subunit within the 19S complex. Attention is given to determining the catalytic properties of the six ATPase subunits and probing ATP-dependent conformational changes of the complex.

20S CP ubiquitin-independent degradation

It is widely accepted that proteasomal substrates must be ubiquitinated for recognition and unfolding by the 19S RP before they can be degraded by the 20S CP. A new concept has recently emerged, suggesting that proteins harboring natively unstructured regions undergo "default" degradation in an ubiquitin-independent mechanism which is mediated only by the CP unit. It was recently predicated that between 36 to 63% of the eukaryotic proteins contain intrinsically disordered regions under physiological conditions (Tsvetkov et al, 2007), and tend to form intercellular aggregates (Watanabe et al, 2001). Given that aggregated proteins are a common cytopathological feature of neurodegenerative diseases (Chung et al, 2001; Mather et al, 1993; Watanabe et al, 2001), it is interesting to speculate that the 20S ubiquitin-independent proteasomal degradation pathway is a key player in maintaining normal cell functions and viability by degrading unfolded proteins.

The existence of a distinct 20S ubiquitin-independent mechanism for protein degradation is very intriguing and raises many fundamental questions: How is this process regulated? Are there mediating proteins which guide substrates to the 20S? What triggers gate opening and entry to the catalytic chamber? Are there any "protective" proteins which shield intrinsically unstructured proteins from degradation? We are applying structural MS approaches to reveal the molecular mechanism of the RP-independent degradation.

ER-Associated Degradation

Misfolded proteins and 'orphan' subunits of oligomeric complexes may account for up to 30% of newly synthesized proteins in the ER (Schubert et al, 2000). Such aberrant proteins are deleterious to the cell and therefore must be eliminated (Glickman & Ciechanover, 2002). Inefficient ER-associated degradation results in accumulation of misfolded proteins in the ER and triggers cell death. Dysfunctional degradation mechanism of misfolded proteins is also reported to be involved in diabetes and neurodegenerative diseases such as Alzheimer's and Parkinson's (Hoozemans et al, 2005; Imai et al, 2000; Katayama et al, 1999; Unterberger et al, 2006).

In the mid-1990s it was discovered that misfolded ER-derived proteolysis substrates are degraded by the same machinery that is responsible for the removal of misfolded cytosolic proteins. Therefore, the misfolded ER proteins must first be retro-translocated or 'dislocated' into the cytosol before they can be eliminated by the ubiquitin–proteasome system. This pathway is referred to as "ER-associated degradation" (ERAD) (McCracken & Brodsky, 1996). However, the pathway by which ER-substrates are targeted back to the cytosol and access the

proteasome are not fully understood. We are applying protein complex affinity purification techniques together with structural mass spectrometry to shed light on this process.

Functional Modules of the COP9 Signalosome Complex

The COP9 signalosome (CSN) is a conserved multifunctional complex (Chamovitz et al, 1996; Wei et al, 1994; Wei et al, 1998), containing eight core subunits. Each of its subunits is evolutionary related to the 19S lid subunits (Glickman et al, 1998). The CSN was implicated to be involved in many biological processes such as cell-cycle progression, development, signal transduction, DNA repair, transcription, and regulation of multiple cancers (Cope & Deshaies, 2003; Harari-Steinberg & Chamovitz, 2004; von Arnim, 2003). Recent evidence from several systems indicates the possible existence of CSN sub-complexes ('mini'-CSN), (Mundt et al, 2002; Oron et al, 2002; Tomoda et al, 2002), but the functional relevance of these 'mini'-CSN complexes has not been demonstrated. We are focusing on the composition and mode of action of the CSN sub-complexes versus the intact holocomplex.

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