doi:10.1017/S1431927613002341
Microsc. Microanal. 19 (Suppl 2), 2013

Structure of the Human 26S Proteasome

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In eukaryotes the ubiquitin/proteasome pathway is responsible for the controlled targeting and degradation of a wide range of proteins, including key cellular regulators such as those controlling cell cycle progression and apoptosis. The 26S proteasome is a large multi-subunit ATP dependent protease complex of approximately 2.6 MDa that is responsible for the highly regulated proteolysis of proteins targeted for breakdown by ubiquitin conjugation [1]. It consists of a 20S core containing the proteolytic active sites and two 19S regulatory particles (19S-RP). The 19S-RPs are responsible for the recognition, unfolding and translocation of substrate proteins into the proteolytic core. The 26S proteasome is a well-established target for cancer therapy and its deregulation is associated with neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. Nevertheless, the functional mechanisms of this fundamental protein complex are still largely unknown. In order to address this issue the full understanding of the complete structural organisation of the 26S proteasome is required.

We have determined the structure of the human 26S proteasome by cryo-electron microscopy and single particle analysis. The sample of human 26S proteasome was diluted in buffer containing 2 mM ATP and 5 mM $MgCl_2$ and applied to 1.2/1.3 Quantifoil grids covered with an extra layer of freshly floated continuous thin carbon. The grids were flash frozen using a Vitrobot (FEI), transferred into a FEI TF20 electron microscope and images recorded using a Tietz F415 CCD camera. A data-set of 12,718 particle images was assembled manually using the EMAN Boxer interface. The image analysis was performed using a combination of IMAGIC, Spider and Tigris software, together with some in-house developed software. The resulting 3D maps were visualised using Pymol. The fitting of atomic coordinates and models was refined using URO software.

We obtained a map of the human 26S proteasome to a resolution of 7-9Å [2]. We clearly recovered the secondary structure for most of the 20S core sub-complex. The six AAA-ATPase Rpt subunits, responsible for the unfolding of the substrate proteins, form a rather asymmetric hetero-hexameric assembly immediately adjacent to the 20S core, although these two sub-complexes are neither co-axial nor co-planar. Rpn1 and Rpn2 are the largest subunits of the 26S proteasome (~100 kDa) and have been associated with substrate recognition. The Morris lab determined the structure of Rpn2 by x-ray crystallography, revealing a novel fold for its PC domain [3], which allowed its unequivocal location within the 26S proteasome complex. This provided the basis for the assignment of all remaining subunits of the complex and allowed the fitting of available crystallographic structures or structural models for each of the subunits into their corresponding densities, resulting in a molecular model for the complete human 26S proteasome (Figure 1) [2].

The current map of the human 26S proteasome serves as a basis for the complete understanding of its structural organisation. Interestingly, the subcomplex formed by the PCI-MPN subunits of the

19S-RP form an assembly with a structural organisation that is remarkably similar to that of another regulator of the ubiquitin/proteasome pathway, the COP9/signalosome, for which a low resolution map is now available [4], although the functional consequences of this structural conservation are still largely unknown. Overall, the information now available on the structure of the human 26S proteasome serves as a strong framework for further experimental work that should lead to a full understanding of the functional mechanisms of this fundamental protein complex.

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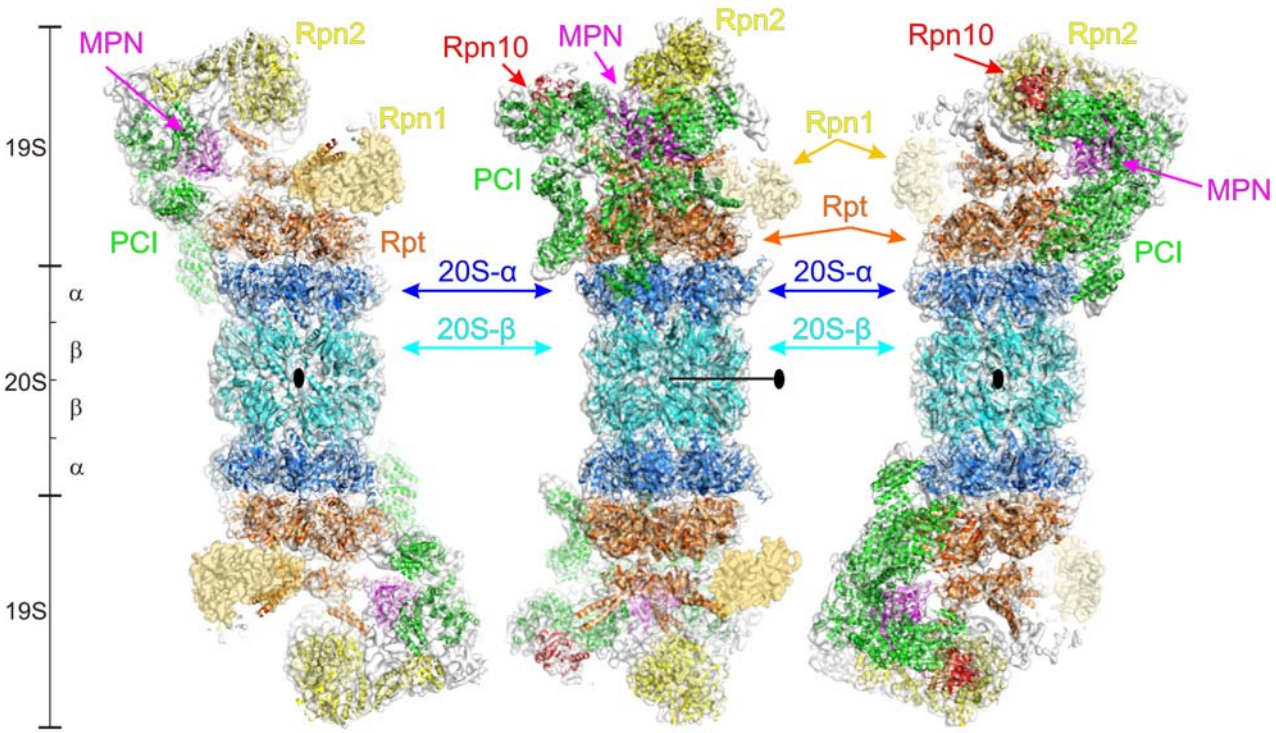

Figure 1. Molecular model of the human 26S proteasome. Orthogonal views of the human 26S proteasome map (transparent surface) with fitted coordinates for all its main subunits (cartoon representations). All families of subunits, for both the 20S core and the 19S-RP, are identified and colour-coded.

https://doi.org/10.1017/S1431927613002341 Published online by Cambridge University Press