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Structural insight into proteasome orchestration controlled by assembly chaperone Nas2

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1 Introduction

The proteasome plays an indispensable role in degrading proteins conjugated to ubiquitin. This enzyme is a huge proteolytic machine and comprises a catalytic core particle (CP) and one or two 19S regulatory particles (RPs). Recently accumulated evidence revealed that the proteasome orchestration does not occur due to spontaneous self-organization but results from a highly ordered process assisted by several "assembly chaperones". The assembly of the ATPase subunits of RP is assisted by four assembly chaperones (Hsm3, Nas6, Rpn14, and Nas2). To date, the three-dimensional structures of the RP-assembly chaperones have been determined except for that of Nas2. Although these studies provided some structural insights into proteasome assembly, the structural basis for the functional role of Nas2 remains unknown. The aim of this study is to provide the key missing link in the proteasome assembly pathway [1].

2 Experiments

The bioinformatic analysis of the Nas2 showed that this assembly chaperone consists of N-terminal helical and C-terminal PDZ domains (termed Nas2N and Nas2C hereafter) connected by a flexible linker. To examine the contributions of the individual domains to Rpt5C interaction, we performed the several biochemical and biophysical experiments using the isolated domains. Consequently, we revealed a dual recognition mode for Rpt5C binding by the two domains of Nas2: the Nas2N mainly mediates the interaction with Rpt5C, which is reinforced by the Nas2C.

Based on the result, we attempted to crystallize full-length Nas2 and Rpt5C complex. However, despite extensive crystallization screening, the complex crystals could not be produced. Therefore, we performed the crystallization using Nas2N and an Rpt5 chimera (PAN~Rpt5C), which is composed of an N-terminal ATPase domain from the Rpt archaeal homolog (PAN) and a C-terminal helical domain from yeast Rpt5. Through this approach, we solved the complex structure of Nas2N and PAN~Rpt5C at 4.0 Å resolution. During the structural determination processes, uncomplexed Nas2N and PAN~Rpt5C structures were also determined at 1.65 Å- and 2.6 Å-resolution, respectively. The diffraction data sets were collected at Photon Factory AR-NE3A, SPring-8 BL44XU, and NSRRC 13B1 beamlines.

3 Results and Discussion

The crystallographic data revealed that Nas2N formed a 1:1 complex with Rpt5C (Fig. 1). Intestingly, the Nas2N–Rpt5C complex has a distinct binding mode in comparison with the other structurally characterized RP-assembly chaperones, i.e., Hsm3, Nas6, and Rpn14. Binding of these three assembly chaperones to RP is compatible with the formation of an Rpt hexameric ring without steric clashes. In contrast, binding of the Nas2N to Rpt5 causes significant steric hindrances against neighboring Rpt1-2 complex. Using NMR spectroscopy, we also demonstrated that Nas2C interacts with the C-terminal proteasome-activating motif of Rpt5C.

In summary, we revealed that Nas2 interacts with the proteasome activator Rpt5 subunit in a bivalent mode: the N-terminal domain of Nas2 masks the neighboring Rpt1-interacting surface of Rpt5, whereas its C-terminal PDZ domain caps the proteasome-activating motif. Therefore, Nas2 serves as a proteasome activation blocker, providing a checkpoint during the formation of the proteasome ATPase ring prior to its docking onto the catalytic CP.

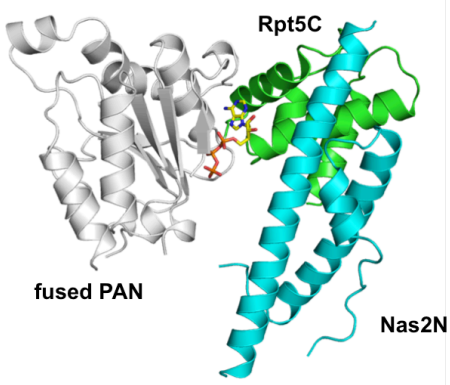

Fig. 1. Crystal structure of Nas2N/Rpt5C complex

References

[1] T. Satoh *et al*., *Structure* **22**, 731-743 (2014).

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