

MS7-P11 Structural similarity between the N-terminal domain of LonA proteases and the highly conserved RNA-binding PUA domainsAlla Gustchina¹, Alexander Wlodawer¹, Tatyana Rotanova²

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Homooligomeric LonA proteases are the key components of the protein quality control system in bacteria and eukaryotes. Proteolytic activity of LonA is coupled to ATP hydrolysis. Pioneering studies in the 1980s have shown that members of this family of proteases/ATPases are also nucleic acid-binding proteins, and their proteolytic and ATPase activities are stimulated by DNA binding. Such studies indicated that a number of different DNA species increased the rate of degradation of target proteins by the protease, and suggested that association with DNA might be involved in regulation of protein breakdown in cells. In particular, studies of the mitochondrial LonA demonstrated that this enzyme binds to DNA and RNA, and that the binding affinity is affected by the presence of a nucleotide and a protein substrate.

Structural data for the individual domains and/or their combinations have recently become available for several representative LonA proteins. Crystal structure of the N-terminal fragment of *E. coli* LonA comprising residues 1-117 revealed structural similarity to the PUA domain, a highly conserved RNA-binding motif found in a wide range of archaeal, bacterial, and eukaryotic proteins. Here we compare the structure of the N-terminal domain of LonA to the structures of the PUA domains from several protein complexes with RNA, with the aim to reveal possible epitopes for the interactions with nucleic acids.

Keywords: ATP-dependent protease, structure comparison**MS7-P12** Structural investigation of importin β :importin7:histone1 complexNives Ivic¹, Silvija Bilokapic Halic¹, Mario Halic¹

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During DNA replication, histones synthesised in the cytoplasm must be imported into the nucleus for the formation of nucleosomes on newly replicated DNA. In spite of their small size, histones are transported by active transport requiring import receptors. The core histones are transported in the nucleus by monomeric import receptors, members of the importin β superfamily. Exceptions are linker histones, which require a formation of a heterodimeric receptor consisting of two importin β family members, importin β (Imp β) and importin7 (Imp7). Up to date, there is no detailed structural information about the interaction network and recognition mode between importin β , importin7 and histone1. Biochemical data indicate that both, histone1 core and tail, are important for the interaction with Imp β :Imp7, which should result with defined histone tail structure. Each of the proteins is individually overexpressed in bacterial cells and for all of them a successful purification protocol has been established. The importin β :importin7:histone1 complex was prepared by mixing the three proteins in a molar ratio 1:1:1 in assembly buffer. Currently crystallization trials are underway. With molecular size of around 250 kDa, the trimeric complex is at the limits of cryo electron microscopy, which makes it a very good model for pushing cryoEM to new frontiers. Initial negative stain grids have been prepared for each component of the complex, as well as the complex itself. The grids have been inspected using the electron microscope Morgagni. Complex particles can be clearly distinguished from the single particles found on the grids. Negative stain data collection for initial 3D reconstruction will be performed; as well the samples for cryo-electron microscopy (cryoEM) are being prepared.

Keywords: protein crystallography, histones, importins, nucleosome