CryoEM Structures of Metazoan AAA Protein Bcs1L Captured in ATP Hydrolysis Suggested a Concerted Membrane Translocation Mechanism of Folded Substrate

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AAA (ATPases Associated with various cellular Activities) proteins are molecular machines that are ubiquitously present in all kingdoms of life, performing essential functions in diverse cellular pathways from protein homeostasis, membrane fusion, DNA replication to substrate translocation. They are frequently hexameric with each subunit consisting of an N-terminal functional domain followed by one or two AAA ATPase motor domains. Recent cryo-EM analysis of various AAA proteins in the presence of substrates revealed that AAA proteins engage substrates ubiquitously by the threading mechanism, in which subunits of AAA proteins arrange in a spiral staircase formation with each subunit captured in action interacting with an amino acid of protein peptide or a base of DNA in a hand-over-hand fashion. Importantly, subunits of various nucleotide states (non-uniform nucleotide states), apo, ADP or ATP, coexist in the same protein.

The metazoan AAA-ATPase Bcs1L translocate the fully assembled Rieske iron-sulfur protein (ISP) precursor across the mitochondrial inner membrane, facilitating assembly of the respiratory Complex III. Recent structures of mouse Bcs1L and yeast Bcs1 revealed heptameric association of Bcs1 subunits and substantial changes in size of the proposed substrate-binding cavity under different bound nucleotide states, suggesting a mechanism for protein cargo movement [1,2]. However, it remains unclear structurally the binding of folded substrate to and release from Bcs1 and the question persists whether subunits of Bcs1 act in sequence or in unison when moving the protein cargo. Here, we captured mouse Bcs1L conformations by Cryo-EM analysis while it is actively hydrolyzing ATP in the presence or absence of the ISP substrate. In contrast to the hand-over-hand mechanism widely adopted by known AAA proteins, our results show that subunits of heptameric Bcs1L transit uniformly between ATP and ADP conformations with no co-existing different nucleotide states, which is in favor of Bcs1 subunits acting in a concerted manner. We further show that ISP can be trapped when subunits of Bcs1L is uniformly in the ADP-bound state, and it could be released laterally in the apo form.

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