

The Crystal Structure of the MJ0796 ATP-Binding Cassette: Implications for the Structural Consequences of γ -Phosphate Hydrolysis in the ATPase Active Site of an ABC-Transporter

Y. Yuan, S. Blecker, O. Martsinkevich, L. Millen, P. Thomas, and J.F. Hunt (Columbia U.)

Abstract No. yuan8829

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Introduction: ATP-binding cassette (ABC) transporters are mechanochemically coupled polypeptide complexes responsible for transmembrane solute translocation against cellular concentration gradients. In prokaryotes and archaeobacteria, these complexes are generally composed of four subunits, two of which are membrane-embedded proteins with 6 transmembrane α -helices each that are thought to determine substrate specificity and trajectory. The other two subunits are peripherally associated with the membrane-spanning components and mechanically couple ATP hydrolysis to solute translocation. In higher eukaryotes, these components are fused into one polypeptide containing multiple domains and have been linked to a number of human diseases, the most notable of which are cystic fibrosis and multidrug resistance in advanced tumor cells.

As the individual domains are present as separate genes for many of the prokaryotic transport systems, the roles of the different domains can be studied individually. Therefore, we sought to identify the structural substrates of an ATP-binding cassette from an ABC transporter in order to relate the structure of these intermediates to their role in the active transport reaction cycle. We solved the X-ray crystal structure of the ATP-binding cassette from the LoiD ABC transporter from *M. jannaschii*, LivF, bound to MgADP.

Conclusions: Comparing this structure of that of the ATP-bound form of the HisP ATP-binding cassette shows a 0.5nm withdrawal of a phylogenetically invariant glutamine residue from contact with the γ -phosphate of ATP in the active site. Considering this subdomain movement in the context of the likely physiological dimer of cassettes present in ABC transporters indicates that it produces a modest mechanical change that could play a role in facilitating nucleotide-exchange out of the ATPase active site. One of the intersubunit packing interactions in MJ0796 crystal involves antiparallel β -type hydrogen bonding interactions between the outermost β -sheets leading to their fusion into a single extended β -sheet; as this type of structural interaction has been proposed to play a role in mediating the aggregation of β -sheet-containing proteins like the ATP-binding cassettes, it is interesting to note that both the solvent-accessible surface area buried in this adventitious interstrand interface and also its level of surface complementarity are similar to those in most of the β -strand pairings in the interior of the β -sheets.