

## **Structural Study of a Non-Canonical ABC Importer Complex, the *E. coli* Ribose Transporter (RbsABC)**

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RbsABC is a high affinity, inducible ABC transporter for ribose intake. Based on structural and biochemical properties, RbsABC is a non-canonical substrate protein-dependent ABC importers. The peculiarities of RbsABC present several enigmas in the transport mechanism of ABC importers that were well established previously. Therefore, elucidating how these unique features confer mechanistic differences to the enigmatic RbsABC will provide a new perspective on how ABC importers achieve substrate transport. X-ray crystallography and cryo-electron microscopy (cryo-EM) were exploited to generate structural data that were used to understand this complex system. The crystal structure of RbsABC revealed a channel-like substrate translocation pathway, that was not reported in other ABC importers, lining the RbsC translocation domain. The periplasmic opening was sealed by RbsB and lined by negatively charged residues while the remaining pathway is hydrophobic in nature, suggests a non-measurable binding affinity of the complex towards ribose ('Teflon lining'). Cryo-EM was utilized to understand conformational changes during the ATP-hydrolysis/substrate transport cycle. In this study, Leu255 and Phe127 were identified as periplasmic and cytoplasmic gating residues, respectively. These gating residues occlude the substrate translocation pathway at different stages in the ATP-hydrolysis cycle to achieve vectorial substrate transport via a 'peristaltic pump' mechanism. Based on our structural study, RbsABC more closely resembled the prototype of the type II ABC importer, the vitamin B<sub>12</sub> transporter. However, the ATP-hydrolysis assay revealed that RbsABC ATP hydrolysis is stimulated by substrate-loaded substrate-binding protein, a biochemical signature of type I ABC importer. Synthesizing both biochemical and structural information in this study, RbsABC is a hybrid of type I and II ABC importers and may be a unique class by itself.