Structural basis of trehalose recycling by the ABC transporter LpqY-SugABC

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Abstract

In bacteria, adenosine 5′-triphosphate (ATP)–binding cassette (ABC) importers are essential for the uptake of nutrients including the nonreducing disaccharide trehalose, a metabolite that is crucial for the survival and virulence of several human pathogens including Mycobacterium tuberculosis. SugABC is an ABC transporter that translocates trehalose from the periplasmic lipoprotein LpqY into the cytoplasm of mycobacteria. Here, we report four high-resolution cryo–electron microscopy structures of the mycobacterial LpqY-SugABC complex to reveal how it binds and passes trehalose through the membrane to the cytoplasm. A unique feature observed in this system is the initial mode of capture of the trehalose at the LpqY interface. Uptake is achieved by a pivotal rotation of LpqY relative to SugABC, moving from an open and accessible conformation to a clamped conformation upon trehalose binding. These findings enrich our understanding as to how ABC transporters facilitate substrate transport across the membrane in Gram-positive bacteria.

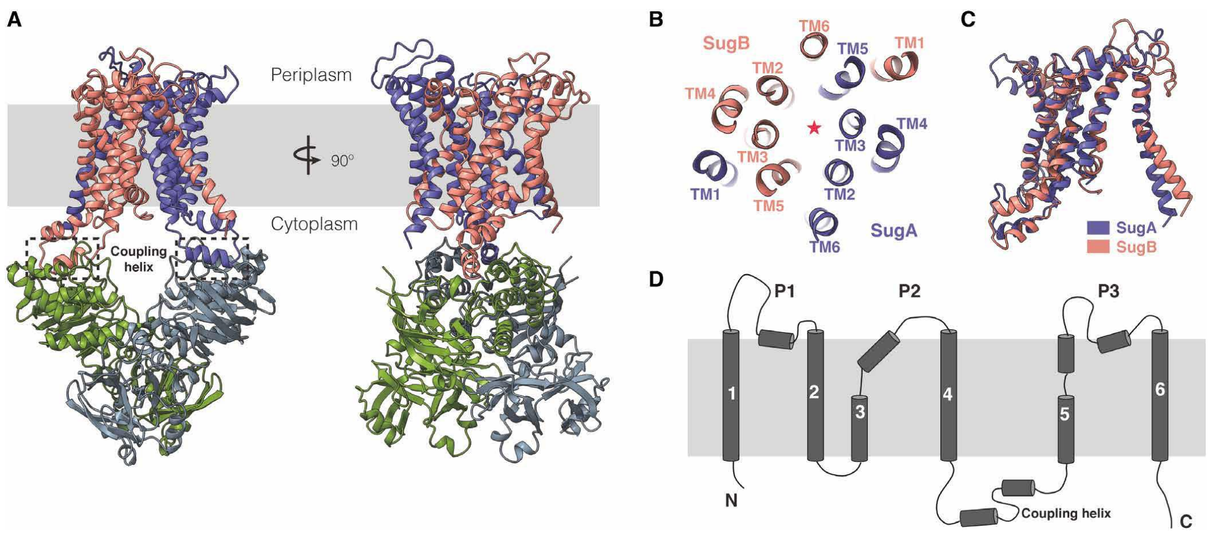

Fig. 1. The molecular architecture of SugABC. (**A**) Cartoon representation of SugABC viewed from with the membrane represented by the gray box. SugA and SugB are colored violet and peach, respectively. SugC is colored green or gray. The coupling helices in SugA and SugB are also marked by dashed boxes. (**B**) Clipped view of the transmembrane region of SugABC viewed from the periplasm. The star indicates the pseudo-twofold axis. (**C**) Superposition of SugA and SugB structures. (**D**) Topological diagram of SugA or SugB.

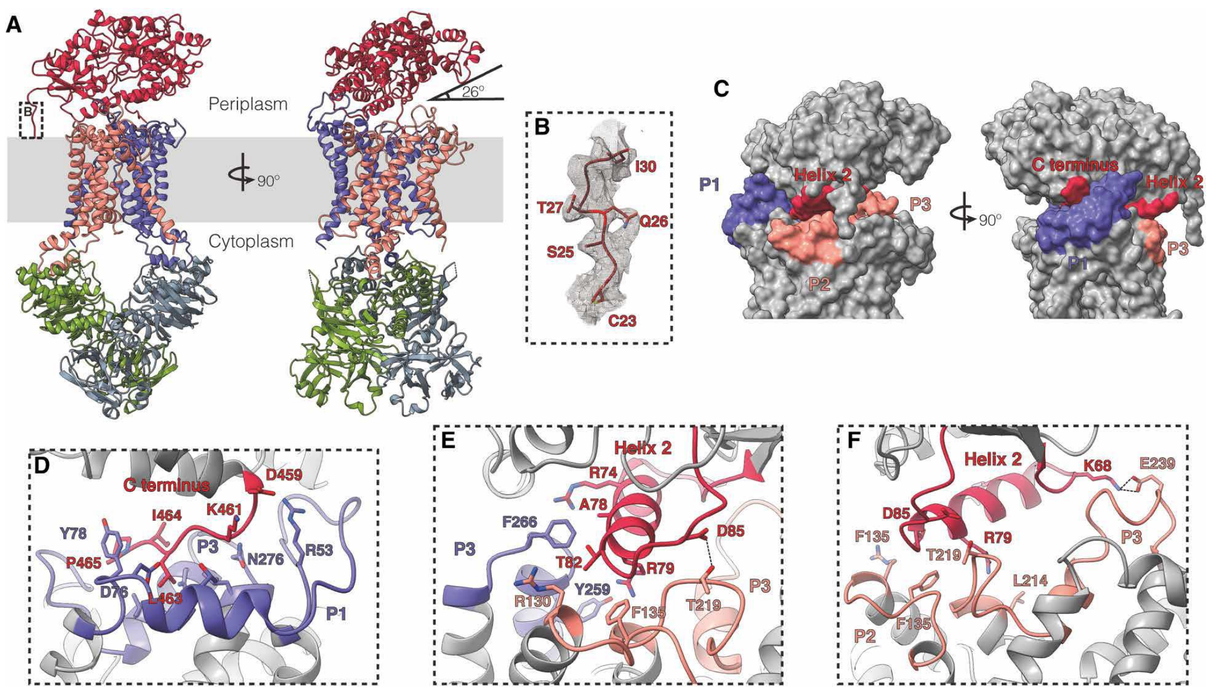

Fig. 2. A unique resting state for SugABC in complex with the lipoprotein LpqY. (**A**) Two views of LpqY-SugABC in the resting state. The lipoprotein LpqY (red) tilts by ~26° relative to the transmembrane region of SugABC, and its N terminus likely inserts into the detergent micelle. (**B**) EM map for the N terminus of LpqY in the resting state. (**C**) Surface representation of the interactions of LpqY with TM region in the resting state. The regions of involved in interaction are colored. The C terminus and helix 2 of LpqY are red. The SugA P1 and P3 loops and SugB P2 and P3 loops are violet and peach, respectively. (**D** to **F**) Details of the interactions shown in (C). Hydrogen bonds and salt bridges are indicated by dashed lines.

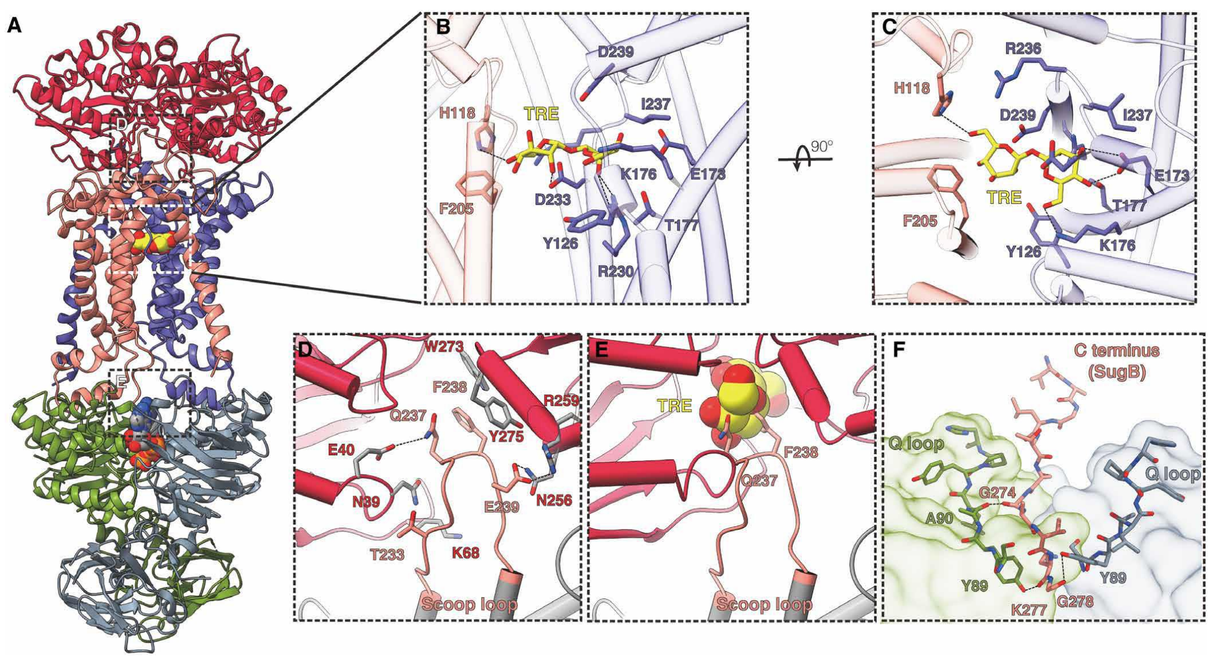

Fig. 4. Structure of the trehalose transporter in the catalytic intermediate conformation. (**A**) LpqY-SugABC bound to trehalose and ATP in the catalytic intermediate state. SugABC has an outward-facing conformation. The two SugC subunits form a closed dimer. Trehalose and ATP are drawn as spheres with yellow and coral carbon atoms, respectively. (**B** and **C**) Two close-up views of the trehalose-binding site in the TM region. Hydrogen bonds are indicated by dashed lines. (**D**) Insertion of the P3 loop (scoop loop) of SugB into the LpqY trehalose-binding pocket. The residues involved in the interactions with the scoop loop are shown as gray sticks. Hydrogen bonds and salt bridges are indicated by dashed lines. (**E**) Insertion of the SugB scoop loop into the substrate-binding site of LpqY. A trehalose molecule is modeled into the binding site on the basis of the structure of LpqY-SugABC bound trehalose in the pretranslocation state. (**F**) Insertion of the C-terminal segment of SugB into the SugC dimer interface. The residues involved in interactions are shown as side-chain sticks. Hydrogen bonds are indicated by black dashed lines.

Fig. 3. Conformational changes of LpqY in LpqY-SugABC upon trehalose binding.
(**A**) LpqY-SugABC–bound trehalose structure in the pretranslocation state. Trehalose (yellow spheres) is located in the substrate-binding pocket of LpqY. SugABC has an inward-facing conformation in this state. (**B**) Conformational changes of LpqY upon trehalose (TRE) binding. LpqY-SugABC in the resting state (left) and pretranslocation state (i.e., after trehalose binding) (right). (**C**) Binding of trehalose to LpqY in the pretranslocation state. Trehalose and the residues involved in interactions are shown in yellow and gray sticks, respectively. Hydrogen bonds are indicated by dashed lines. (**D**) Atomic model of trehalose superimposed with the cryo-EM density in the pretranslocation state. (**E**) Surface representation of LpqY before and after trehalose binding. The cleft closes upon trehalose binding. Trehalose is shown as yellow and red spheres. The surface of the trehalose bound structure is shown in transparent mode.

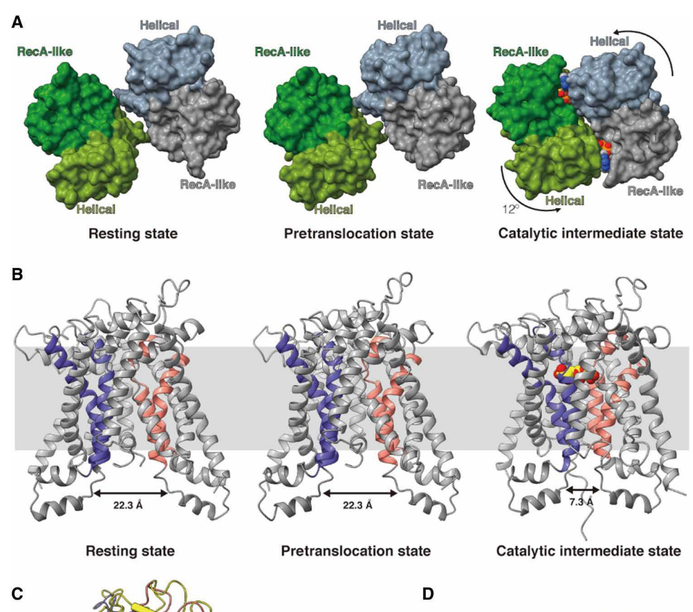

Fig. 5. Conformational changes of LpqY-SugABC upon ATP binding. (**A**) Surface representations of the NBDs in the three states viewed from cytosol. The RecA-like domain and the helical subdomain are differentiated by color shadings. The rotation of the helical subdomain with respect to the RecA-like domain during transition from the pretranslocation state to the outward-facing conformation is indicated. (**B**) Comparison of the transmembrane domains of LpqY-SugABC in different states. The key TM helices 4 and 5 of SugA and SugB are colored violet and peach, respectively. The coupling helices are colored gray. The distances between the two coupling helices are indicated. (**C**) Left: Superposition of TM region of LpqY-SugABC in the pretranslocation (colored yellow) and catalytic intermediate states based on the SugA subunit (SugA and SugB colored violet and peach, respectively). Right: Conformation comparison of TM5 helix in the two states. (**D**) Shift of TM helices viewed from membrane. The helices are shown as cylinders colored in violet (SugA) and peach (SugB) in the catalytic intermediate state. Corresponding helices in the pretranslocation state are colored in gray.

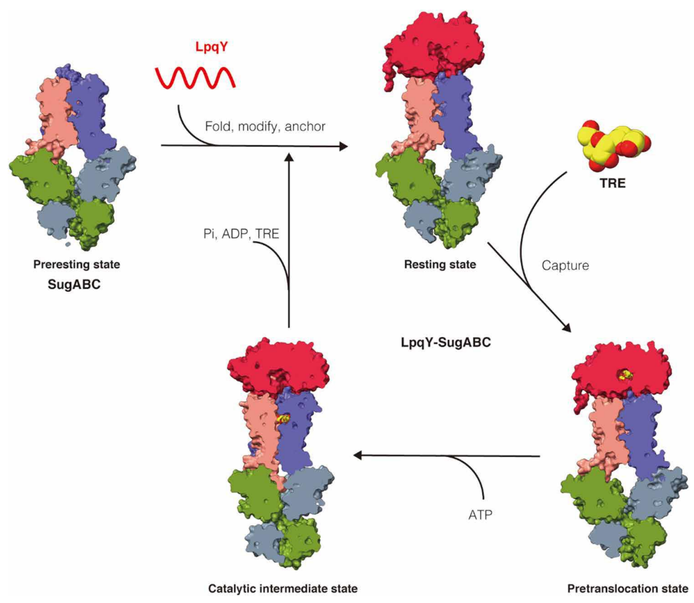

Fig. 6. Schematic of proposed trehalose transport mechanism. The trehalose transporter LpqY-SugABC is proposed to transport trehalose by cycling through a trehalose-free state (the resting state), trehalose-bound state (pretranslocation state), and ATP-bound state (catalytic intermediate state). In the resting state, LpqY leans against the TM region of SugABC in an open form. Free trehalose is captured by LpqY, while LpqY docks onto the TM region of SugABC (pretranslocation state). ATP binding causes SugC to assume a closed conformation, coupled to the conformation changes of the TM region and LpqY. As a result, trehalose transfers into a translocation cavity in the TM region. Upon ATP hydrolysis and phosphate release, trehalose is released into the cytoplasm. Subsequently, the transporter LpqY-SugABC reverts back to the resting state.

Acknowledgments:

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