Crystal structure of YidC reveals a mechanism of Sec-independent membrane protein

The plasma membrane, which is mainly composed of phospholipids and membrane proteins, works as a boundary between the inside and outside of living cells. A variety of membrane protein functions in processes that take place within and across the membrane, such as energy generation, signal transduction, and ion transport. These membrane proteins are synthesized in the cytoplasm and are then inserted into the membrane by protein machineries. The YidC/Oxa1/Alb3 family proteins play key roles in protein insertion into bacterial, mitochondrial and thylakoidal membranes, respectively.

In the bacterial inner membrane, YidC inserts proteins into the membrane on its own, facilitates proper folding of membrane proteins inserted by a protein-conducting channel, the SecYEG translocon, and is essential for cell viability [1,2] (Fig. 1). Recent studies have revealed that Sec translocon possesses an aqueous channel for translocation of hydrophilic peptides across the membrane as well as membrane protein insertion. In contrast, the lack of high-resolution structures of YidC hampers the understanding of the molecular mechanisms for membrane protein insertion mediated by YidC. Here, we determine the crystal structure of YidC from *Bacillus halodurans* at 2.4 Å resolution by the multiwavelength anomalous diffraction method using a mercury-derivatized crystal [3,4]. All diffraction data were collected at beamline **BL32XU**.

The YidC structure consists of an N-terminal helix (EH1), two cytoplasmic helices (CH1 and CH2), and a core region composed of five transmembrane helices (TM1–5), which reveal a novel protein fold (Fig. 2(a)). The EH1, CH1, and CH2 helices lie parallel to the membrane surface. The amphipathic EH1 helix is partly embedded in the membrane, while the CH1 and CH2 helices are composed of mainly polar or charged residues that are fully exposed to the cytoplasm. The TM1–5 helices form a hydrophilic groove in the cytoplasmic leaflet, which is open to both the membrane interior and the cytoplasm, but is closed on the extracellular side by the hydrophobic core of the protein (Fig. 2(b)). This groove contains many hydrophilic residues (Fig. 2(c)), generating a hydrophilic environment that spans the cytoplasmic leaflet of the membrane. In particular, the conserved arginine, Arg72, is present in the center of the groove, which results in a positive electrostatic potential on the groove surface (Fig. 2(b)). Molecular dynamic simulations show that the groove can accommodate about 20 water molecules, but the water molecules do not permeate the membrane during a 1-μs simulation. These observations suggest that YidC, unlike the Sec translocon, does not possess a pore for protein translocation.

To investigate the functional importance of the hydrophilic groove, we performed a genetic analysis of SpoIIIJ, the YidC orthologue in *Bacillus subtilis* using a β-galactosidase assay to monitor the membrane insertion of MifM [5], a substrate protein that is inserted in a SpoIIIJ-dependent manner. Substitution of the arginine, Arg73 in SpoIIIJ, with an alanine abolished MifM insertion activity, while substitution of other conserved glutamines in the groove with an alanine did not affect the activity. In contrast, a lysine substitution of the arginine retained its partial activity. These results suggested that a positively charged residue is important in the hydrophilic groove in order for YidC activity to act as an insertase. MifM is a single-spanning membrane protein, possessing three positively charged residues in the N-terminal extracellular regions. Next we mutated the positively charged residues to neutral residues and assessed the membrane insertion of the MifM mutants. The results showed that these mutations negatively

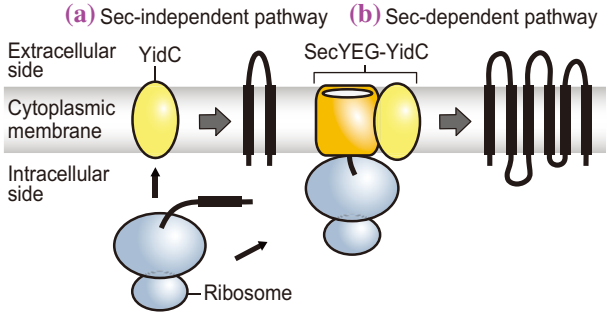

Fig. 1. YidC plays two different roles in membrane protein insertion. **(a)** YidC mediates membrane protein insertion on its own. **(b)** YidC works as a membrane chaperone in cooperation with Sec translocon to facilitate membrane protein folding and assembly.

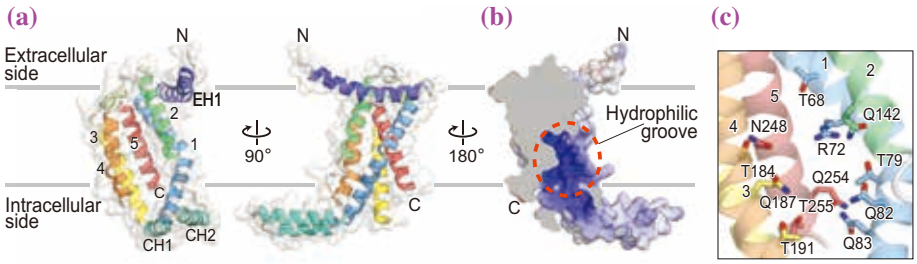

Fig. 2. Crystal structure of YidC. **(a)** Cartoon model of YidC, colored blue to red from the N to C terminus; with the surface model (gray) **(b)** Cross-sectional view of the hydrophilic groove. Surface is colored by the electrostatic potential, ranging from blue (+20 kT/e) to red (–20 kT/e). **(c)** Close-up view of the hydrophilic groove, showing the side chains of hydrophilic residues.

affected the membrane insertion efficiency of MifM, supporting the notion that the positive charge in the hydrophilic groove can attract the negative charge of the substrate protein. This assertion is also supported by *in vivo* site directed photo-crosslinking analysis, which shows direct interactions between the hydrophilic groove and MifM. Altogether, these results suggest that the hydrophilic groove plays a key role in membrane protein insertion as a substrate binding site.

On the basis of the crystal structure and the genetic and biochemical analyses, we propose the following model for membrane protein insertion mediated by YidC (Fig. 3). First, YidC interacts with the extracellular region of the substrate using the hydrophilic groove, which transfers the extracellular region into the membrane. Then the extracellular region is released from the groove and fully translocated across the extracellular leaflet. Next the transmembrane region of the substrate is inserted into the membrane. It should be noted that membrane protein insertion could be promoted by various factors, such as the electrostatic forces by the membrane potential and the hydrophobic interaction between the transmembrane region and the membrane. Overall, our findings pave the way for further investigations into the molecular mechanism of YidC.

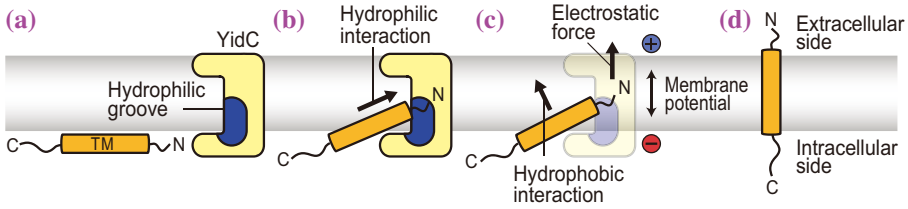

Fig. 3. Model for membrane protein insertion mediated by YidC. Schematic representation of membrane insertion of a single spanning protein that possesses an N-terminal extracellular tail, such as MifM. **(a, b)** Hydrophilic groove of YidC captures the N-terminal tail of substrate, which transfers the N-terminal tail into the membrane. **(c, d)** Subsequently, the N-terminal tail is translocated across the extracellular leaflet while the transmembrane region (TM) is released into the membrane, which could be facilitated by the hydrophobic interaction and the membrane potential.

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