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Projection Structure of yidC: A Conserved Mediator of Membrane Protein Assembly

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Bacteria, mitochondria and chloroplasts harbour factors that facilitate the insertion, folding and assembly of membrane proteins. In *Escherichia coli*, yidC is required for membrane insertion, acting in both a Sec-dependent and a Sec-independent manner. There is an expanding volume of biochemical work on its role in this process, but none so far on its structure. We present the first of this class of membrane proteins determined by electron cryomicroscopy in the near-nativelike state of the membrane. yidC forms dimers in the membrane and each monomer has an area of low density that may be part of the path transmembrane segments follow during their insertion. Upon consideration of the structures of yidC and SecYEG, we speculate on the nature of the interfaces that facilitate the alternative pathways (Sec-dependent and -independent) of membrane protein insertion.

Keywords: protein translocation; membrane insertion; yidC; electron microscopy; projection map

Membrane proteins require the assistance of several protein factors for their correct localisation, insertion and folding. They carry sequences (transmembrane (TM) domains or signal sequences) that target them to specific membranes where they are recognised by protein assemblies responsible for their translocation. This reaction is best understood in a ubiquitous process that occurs in the endoplasmic reticulum (ER) of eukaryotes and the plasma membrane of bacteria and archaea. The respective Sec61 and SecY complexes form gated protein channels in the membrane capable of transferring proteins either across the membrane or into it. This can be achieved by either post- or cotranslational pathways.

In bacteria, the posttranslational translocation route relies on a chaperone SecB and an ATPase SecA,[1] while the cotranslational route utilises the signal recognition particle (SRP) and its receptor.[2] The pathways converge on the translocon and discriminate the substrates according to their signal sequences and hydrophobicity; membrane proteins tend to be directed to the SRP pathway, whereas secreted proteins require the SecB–SecA route.[3–5]

Protein translocation occurs through one monomer of the two found in the active dimeric SecYEG complex.[6,7] Two crystallographic studies have described the structure of the protein channel. One of them visualised the *Escherichia coli* membrane-bound dimer[8] and the other a detergent-solubilised monomer from *M. jannaschii* in atomic detail.[9] The structures identify a lateral gate adjacent to the lipid phase for the entry of TM segments into the bilayer.[8–10]

Several *E. coli* membrane proteins have been shown to require a protein called yidC for their insertion. It contains six predicted TM segments with a large 320-amino-acid domain exposed to the periplasm; it assists membrane protein insertion either with or without the help of SecYEG.[11–18] Both Sec-dependent and Sec-independent pathways seem to proceed cotranslationally and some studies (but not all) report a requirement for SRP.[15,16,18–22]

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Abbreviations used: SRP, signal recognition particle; 2-D, two-dimensional; ER, endoplasmic reticulum; TM, trans-membrane.

yidC homologues have been identified in mitochondria and chloroplasts where they serve similar roles.[12,23–26] In spite of their essential and ubiquitous function, not very much is known about how they work, in part due to a lack of structural information. Therefore, in order to understand more about this reaction we decided to initiate a project, similar to the one described for SecYEG, to examine the architecture of yidC.

Crystallisation and image reconstruction of yidC

The protein was overexpressed and purified to a high degree (Fig. 1). Two-dimensional (2-D) crystallisation experiments produced yidC lattices incorporated into vesicular and sheetlike membranes (Fig. 1). A comparison of the purified and crystalline samples by SDS-PAGE indicated that the specimen containing the crystals consisted of full-length, nondegraded yidC (Fig. 1).

The samples were analysed by electron cryomicroscopy and the resultant images of uncontrasted 2-D crystals were digitised and processed to yield a 10-Å projection map of yidC—a view of the protein in the plane membrane (Table 1, Fig. 2). The unit cell area (Fig. 2a, outer large parallelogram) of 5800 Å^2 can, by comparison with other membrane proteins, accommodate the two clearly visible yidC dimers (Fig. 2, outlined in continuous and dashed black lines). The monomer (Fig. 2, blue outline) has an area of the right size to contain its constituent six TM segments. The assigned *P*2 crystal symmetry indicates that the two different dimers are arranged in an anti-parallel fashion with respect to the membrane plane (Fig. 2a and c), each monomer within the dimer having the same orientation within the membrane (Fig. 2c). Immunogold staining identified identical and specific epitopes on both sides of the membrane (Fig. 3), consistent with the described orientation (Fig. 2c). The two dimers are related to one another by a noncrystallographic symmetry element, a 180° screw axis in the plane of the membrane, which is not a function of the *P*2 space group. This symmetry has not been imposed on the structure and accounts for the two dimers slightly different appearance.

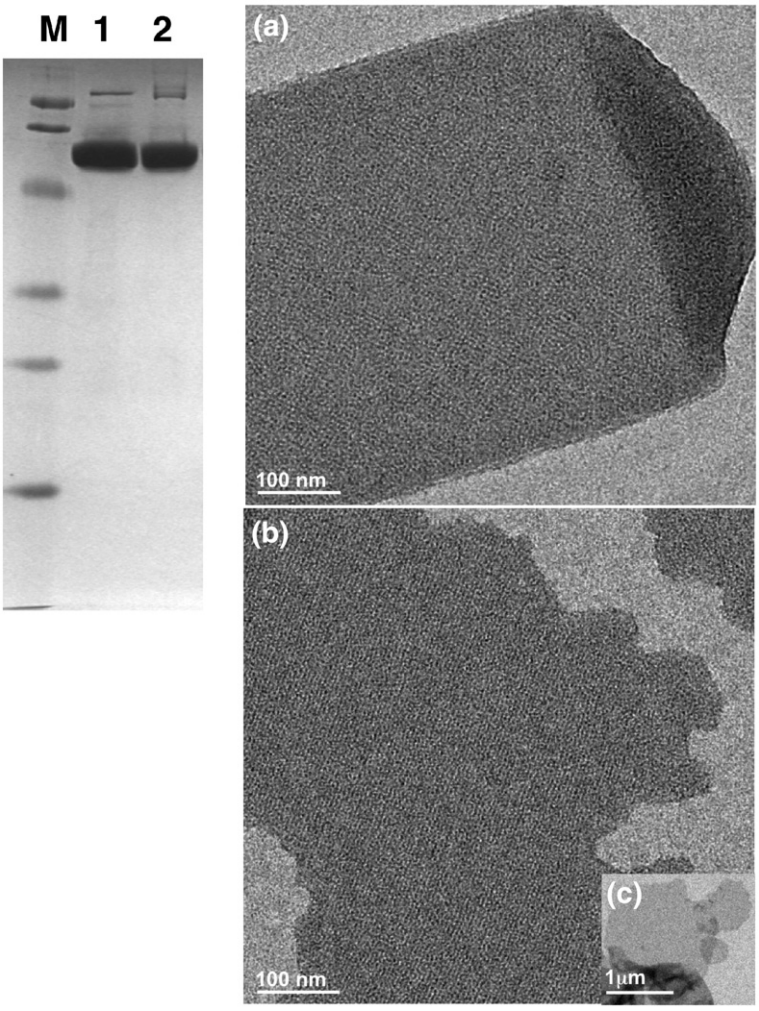

Fig. 1. Purification and crystallisation of yidC. The left panel shows a denaturing gel of purified yidC (lane 1), yidC specimen containing crystals (lane 2) and molecular mass markers (M; 108, 90, 51, 36, 29 and 21 kDa). The right-hand panels show 2-D crystals stained with uranyl acetate. (a) Tubular crystals. (b and c) Crystal sheets. *E. coli* c43 cells[27] harbouring the yidC construct (pBAD/*Myc*-His, Invitrogen) were grown by shaking at 37 °C in 2×YT broth containing 100 μg/ml ampicillin. Overexpression was induced with 0.2% arabinose once the cells had reached an exponential phase of growth. After 3 h, the membranes were harvested by centrifugation following cell lysis and extracted with 1% (w/v) Cymal 6 (Anatrace). The protein was purified by Ni-chelating and gel filtration chromatography and isolated in buffer containing 200 mM NaCl, 0.2% (w/v) decyl maltoside, 20 mM N-(2-Acetamido) iminodiacetic acid, pH 5.6. The purified protein (0.5 mg/ml) was mixed with the dipalmitoylphosphatidylglycerol lipid (Avanti lipids) dissolved in 1% (w/v) decyl maltoside [Glycon; lipid/protein ~0.05–0.2 (w/w)] and dialysed against 100 mM NaCl, 2 mM NaN_3, 1 mM ethylenediaminetetraacetic acid, 20 mM N-(2-Acetamido)iminodiacetic acid at pH 5.6 and 30 °C to remove the detergent. The crystals formed following the depletion of NaCl from the samples.