How does the TOM complex mediate insertion of precursor proteins into the mitochondrial outer membrane?

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A multisubunit translocase of the outer mitochondrial membrane (TOM complex) mediates both the import of mitochondrial precursor proteins into the internal compartments of the organelle and the insertion of proteins residing in the mitochondrial outer membrane. The proposed β-barrel structure of Tom40, the pore-forming component of the translocase, raises the question of how the apparent uninterrupted β-barrel topology can be compatible with a role of Tom40 in releasing membrane proteins into the lipid core of the bilayer. In this review, I discuss insertion mechanisms of proteins into the outer membrane and present alternative models based on the opening of a multisubunit β-barrel TOM structure or on the interaction of outer membrane precursors with the outer face of the Tom40 β-barrel structure.

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Introduction

Protein import into mitochondria is essential for organelle biogenesis and, thereby, for eukaryotic cell viability. At the surface of mitochondria, precursor proteins are recognized by the translocase of the outer mitochondrial membrane (TOM complex). This complex is composed of at least seven different subunits (Fig. 1). Tom20 and 70 are the primary receptors, whereas the subunits Tom40, 22, 7, 6, and 5 form the stable TOM core complex (Fig. 1; Dekker et al., 1998; Künkele et al., 1998; Ahting et al., 1999). Tom40 forms the protein-conducting channel of the TOM complex, and theoretical predictions and secondary structure determinations suggest that the protein has a β-barrel topology (Mannella et al., 1996; Hill et al., 1998; Ahting et al., 2001).

In addition to translocating precursor proteins completely across the outer membrane, the TOM complex is also involved in the integration of precursors of outer membrane proteins into the membrane. The mechanism by which the TOM complex facilitates this latter process is only partially understood. The integration of proteins into other membranes of the cell is likely facilitated by a lateral opening of a membrane-embedded translocon (Alder and Johnson, 2004; Johnson and Jensen, 2004; Rapoport et al., 2004). However, as discussed below, a lateral opening of the β-barrel structure is unlikely in light of thermodynamic considerations. Therefore, it is puzzling how the proposed β-barrel topology of Tom40 is compatible with its function in facilitating the insertion of membrane proteins.

Recent studies shed some light on the mechanisms by which the TOM complex facilitates membrane integration of these proteins (Paschen et al., 2003; Wiedemann et al., 2003; Gentle et al., 2004). Precursors of mitochondrial β-barrel proteins were found to cross the outer membrane to the intermembrane space via the TOM channel before their interaction with the TOB–SAM complex, which mediates their insertion into the outer membrane (Fig. 1; Paschen et al., 2003; Wiedemann et al., 2003; Gentle et al., 2004). Thus, precursors of β-barrel proteins are not released into the outer membrane by the TOM complex. These findings still leave an open question: how does the TOM complex mediate the membrane insertion of proteins containing α-helical transmembrane (αTM) segments?

This contribution deals mainly with the mechanism of outer membrane integration of αTM segments. The major topics I address are (1) how other translocons mediate membrane insertion of substrate proteins, (2) structural characteristics of β-barrel proteins and why a lateral opening of such a structure is unlikely, and (3) alternative models for the insertion of mitochondrial outer membrane proteins.

Lateral opening of translocation pores

Lateral opening of the translocation pore is the favored model for Sec-mediated insertion of proteins into the ER membrane and into the inner membrane of bacteria. In these systems, TM helices of integral membrane proteins are frequently postulated to be inserted first into the translocon channel and then leave the channel laterally into the lipid part of the membrane (Alder and Johnson, 2004; Rapoport et al., 2004). Such a lateral opening can be envisioned by rearrangement of the helices lining the translocation pore. The recently determined three-dimensional (3D) structure of the SecYE–Sec61β complex from archaea appears to support this idea (Van den Berg et al., 2004).

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Abbreviations used in this paper: 3D, three dimensional; TOM, translocase of outer mitochondrial membrane; TM, transmembrane.
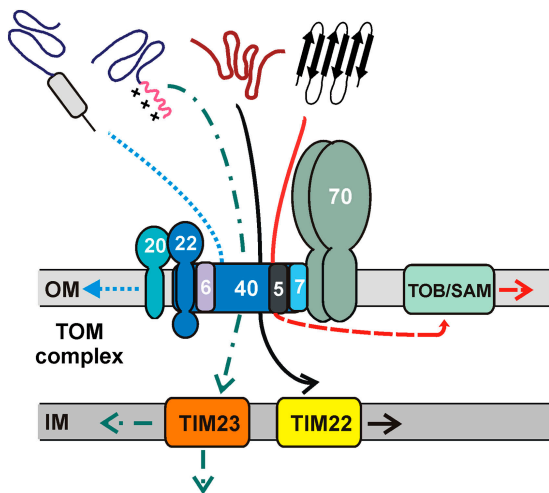
Figure 1. **The TOM complex.** The TOM complex mediates the transloca-tion of mitochondrial precursor proteins. After crossing the outer mem-brane (OM), presequence-containing precursor proteins are engaged by the Tim23 complex in the inner membrane (IM), whereas polytopic inner membrane proteins are inserted by the Tim22 complex. β-Barrel precursors are crossing the outer membrane through the TOM complex and are then reinserted into the outer membrane via the TOB–SAM complex. The TOM complex contains seven subunits: the primary receptors Tom20 and 70 and the TOM core complex, which consists of Tom5, 6, 7, 22, and 40. The names of the Tom subunits reflect their apparent molecular mass.

According to the structure, a lateral gate can be formed at the interface between certain TM helices.

The sorting of mitochondrial inner membrane proteins is also proposed to involve a lateral opening of translocases. Tim23 and Tim22 are two related proteins that are embedded to the inner membrane via four putative TM helices and form the central pore of two distinct translocases in the mitochon-drial inner membrane (Bauer et al., 1996; Sirrenberg et al., 1996). Tim23 is the main component of the translocase for pre-sequence-containing precursors, whereas Tim22 is the central subunit of another complex dedicated to insertion of polytopic inner membrane proteins (Truscott et al., 2001; Rehling et al., 2004). Both proteins are proposed to release TM segments of substrate proteins by lateral opening of their respective chan-nels, yet the precise molecular details remain obscure.

Structural characteristics of β-barrel proteins

In contrast to these insertion machineries, a lateral opening of the TOM complex as part of the insertion mechanisms of pro-teins into the outer membrane of mitochondria is highly ques-tionable (Gabriel et al., 2001; Rapaport, 2002; Johnson and Jensen, 2004). A membrane-embedded β-barrel structure is composed of membrane-spanning antiparallel β sheets that form a closed structure (Wimley, 2003). Such a structure is sta-bilized by a network of nonlocal hydrogen bonds among the β strands. β-barrel membrane proteins are found in the outer membrane of Gram-negative prokaryotic organisms, and the 3D structures of >20 such proteins have been solved so far. Consistent with the endosymbiont theory, which suggests that mitochondria and chloroplasts evolved from endosymbiont

prokaryots, several proteins residing in the outer membrane of these organelles are believed to adopt β-barrel structures. An atomic structure of an eukaryotic membrane β-barrel protein, however, is not yet available.

Recently, the X-ray structure of the 12-stranded au-totransporter serine protease NalP protein revealed that the lumen of its β-barrel domain is large enough to accommodate a single, polar, helical TM that is stabilized through salt bridges and hydrogen bonds with the interior of the barrel wall (Oomen et al., 2004). Even so, as discussed in the next section, it is highly unlikely that β-barrel proteins can release such an ac-commodated TM helix into the lipid core of the membrane.

How does the TOM complex mediate membrane insertion of αTM segments?

Several models can be proposed to explain how Tom40 may be involved in the insertion of outer membrane proteins. For ex-ample, Tom40 as a bundle of α helices would be able to un-dergo a lateral opening without major destabilization of its structure. Although in the absence of the 3D structure of Tom40, this possibility cannot be completely excluded, the fol-lowing evidence supports a β-barrel structure of Tom40: (1) Tom40 is predicted by computer programs to form a β barrel (Mannella et al., 1996); (2) experiments determined β sheets as the major component of its secondary structure (Hill et al., 1998; Ahting et al., 2001); and (3) the biogenesis of Tom40 follows a pathway that is shared by other predicted β-barrel proteins (Paschen et al., 2003; Gentle et al., 2004; Pfanner et al., 2004; Ryan, 2004).

Another unlikely scenario involves a lateral opening of the β-barrel structure (Fig. 2 A). Such a model would require significant displacement of the β strands with respect to each other in order to allow the release of the α helix into the lipid core. However, because the overall structure of the barrel de-pends on interstrand hydrogen bonds, it is hard to envision such a disruption in the network of long-range hydrogen bonds with-out major structural destabilization. It is doubtful that these hy-drogen bonds can be reformed in the low dielectric medium of the membrane. Altogether, this scenario involves the unlikely implication that each insertion of a helical membrane protein results in an irreversible destruction of the β-barrel structure of the translocation pore. Indeed, a lateral opening of any β-barrel structure has not been reported so far.

More favorable scenarios can be proposed for the mecha-nism of membrane insertion. It appears that TOM import receptors are not required for the membrane insertion of pro-teins containing a single αTM close to their NH_2 terminus (Schneider et al., 1991; Suzuki et al., 2002; Ahting et al., 2005). Rather, proteins with αTMs are recognized either directly by membrane-embedded components of the TOM core complex or by a yet unidentified outer membrane protein. A subsequent possible working model is depicted in Fig. 2 B. The two pre-sented pores can be formed by two relatively large β barrels, each of which are composed from β strands that were contrib-uted from three to four different molecules. This possibility re-sembles the β-barrel structure of prokaryotic TolC. The TolC family of envelope proteins is ubiquitous throughout Gram-
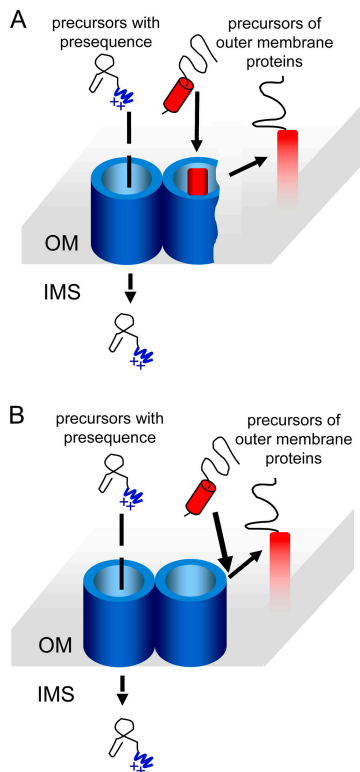
Figure 2. **Is there a lateral opening of the TOM channel?** (A) According to the lateral opening model, the precursor of outer membrane (OM) protein is inserted first into the lumen of the TOM channel. Next, there is a lateral opening of the translocation pore to release the TM segment to the lipid bilayer. (B) In this alternative model, the precursor interacts with the exposed domain of Tom40 and inserts into the interface between the outer face of the complex and the lipid bilayer. IMS, intermembrane space.
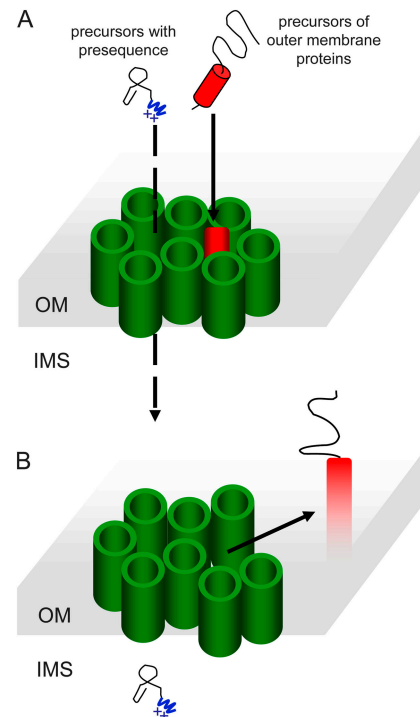

Figure 3. **A working model in which the two pores are surrounded by β-barrel structures.** The TOM complex might contain eight molecules of Tom40. Each of these monomers is in a β-barrel topology, and they are organized in a double-ring structure in which two pores are each surrounded by five Tom40 molecules. Each green cylinder represents one molecule of Tom40. (A) The precursor of outer membrane (OM) protein is inserted first into one of these central pores. (B) Next, there is a rearrangement of the oligomeric structure, resulting in an opening between the subunits, which allows release of the αTM segment to the lipid bilayer. IMS, intermembrane space.

negative bacteria, allowing the export of large protein toxins and proteases and the efflux of small noxious compounds (most notably, antibacterial drugs). The trimeric structure of TolC is composed from a central 12-strand β barrel in which each TolC molecule contributes four β strands to the overall structure (Koronakis, 2003). Interestingly, the interior diameter of the single TolC pore (20 Å) and that of the TOM complex pore are similar (Künkele et al., 1998; Model et al., 2002). In this model, the precursor protein is inserted at the interface between the TOM complex and the lipid phase of the membrane (Fig. 2 B). This model requires neither a lateral opening of the β barrel nor major rearrangement of the complex. In this mechanism, the parts of the Tom40 molecule that are presumed to mediate the insertion of αTM proteins are distinct from the segments forming the general import channel. A recent study that may support this model described a Tom40 variant that is ineffective in the transfer of presequence-containing preproteins but that can sustain normal insertion of outer membrane proteins (Gabriel et al., 2003). This hypothesis is also supported by a recent work on the insertion of outer membrane proteins in chloroplasts. Like Tom40, Toc75 in plants is proposed to mediate both translocation across and insertion into the chloroplast outer membrane and is predicted to be a β-barrel protein (Schleiff et al., 2003). Furthermore, similar to our proposal for the mitochondrial system, Tu et al. (2004) suggest

that two different sites on Toc75 may be involved in binding to either signal sequence-containing precursors or to precursors of outer membrane proteins. Thus, pore-forming components of the translocases of endosymbiont organelles may facilitate distinct modes of protein import into either internal compartments or the outer membrane.

An alternative model presents the TOM complex as an oligomer of eight β barrels surrounding two central cavities that are arranged in a double-ring shape (Fig. 3 A). Taking into account the findings that a dimer is the basic structural element of Tom40 (Dekker et al., 1998; Rapaport et al., 1998), this arrangement would suggest that four dimers are in one complex. The TOM complex was indeed suggested to harbor six to eight molecules of Tom40 (Dekker et al., 1998; Künkele et al., 1998), and electron microscopic visualization of the TOM complex revealed the presence of two to three pores (Künkele et al., 1998; Ahting et al., 1999; Model et al., 2002). Furthermore, several homo-oligomers of β-barrel proteins have been documented (Tamm et al., 2004). In such a model, the translocation pores would be in the center of the ring. According to this scenario, the αTM segment is first inserted into the central cavity of the ring (Fig. 3 A). Next, a rearrangement of the barrels results in the formation of a gate releasing the αTM segment into the lipid bilayer (Fig. 3 B). The reported major rearrange-

ment of the TOM complex as a result of interaction with preprotein suggests that the translocase is indeed a dynamic complex (Rapaport et al., 1998). Such a scenario is appealing because a lateral opening of the β barrel itself is not required. Our observation that a matrix-destined preprotein does not compete with an αTM-containing precursor (Ahting et al., 2005) suggests a model in which the two pores are not functionally equivalent. Rather, matrix-destined precursors are translocated through a distinct pore from the one used by αTM-containing outer membrane proteins. An experimental support for such an assumption and, thereby, for the aforementioned model is currently missing.

The insertion pathway of Bcl-2 into the outer membrane might provide a further example of how to avoid a lateral opening of Tom40. Bcl-2 belongs to a distinct class of proteins that is anchored to the outer membrane by a single TM segment close to the COOH terminus. The mechanism of TM insertion of these tail-anchored proteins is currently unclear. Bcl-2 was reported to be inserted into the outer membrane by a mechanism that involves initial recognition by the import receptor Tom20 but is independent of Tom core components like Tom40, 22, and 5 (Motz et al., 2002). Future studies will tell whether the aforementioned observations, which were performed with mammalian precursor protein (Bcl-2) and yeast import machinery, could be extended to other precursor proteins and to homogeneous systems.

What's next?

Despite considerable progress in our understanding of TOM complex involvement in the biogenesis of mitochondrial outer membrane proteins, further experimental work is required to take us closer to a comprehensive description of this process. Determination of the 3D structure of the TOM complex or of its main component, Tom40, will provide an essential foundation for such future studies. Other important questions regarding the biogenesis of outer membrane proteins include (1) Are membrane-embedded chaperones involved in insertion into the outer membrane and assembly of precursor proteins into functional complexes? (2) Does the specific lipid composition of the mitochondrial outer membrane affect the insertion of outer membrane proteins? (3) Can modifications of substrate proteins and/or Tom subunits regulate the insertion process? Future studies involving a combination of genetic, biochemical, and biophysical methods applied to a broad set of precursor proteins will help us to address these questions.

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