Biochemical Society Transactions (2024)
https://doi.org/10.1042/BST20231236

PORTLAND PRESS

Review Article

New insights into the structure and dynamics of the TOM complex in mitochondria

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To date, there is no general physical model of the mechanism by which unfolded polypeptide chains with different properties are imported into the mitochondria. At the molecular level, it is still unclear how transit polypeptides approach, are captured by the protein translocation machinery in the outer mitochondrial membrane, and how they subsequently cross the entropic barrier of a protein translocation pore to enter the intermembrane space. This deficiency has been due to the lack of detailed structural and dynamic information about the membrane pores. In this review, we focus on the recently determined sub-nanometer cryo-EM structures and our current knowledge of the dynamics of the mitochondrial two-pore outer membrane protein translocation machinery (TOM core complex), which provide a starting point for addressing the above questions. Of particular interest are recent discoveries showing that the TOM core complex can act as a mechanosensor, where the pores close as a result of interaction with membrane-proximal structures. We highlight unusual and new correlations between the structural elements of the TOM complexes and their dynamic behavior in the membrane environment.

Introduction

Since the seminal work of Parsegian co-workers [1] and Kasianowicz et al. [2], the question of how polymers are transported through a natural and artificial pinhole — the so-called art of sucking spaghetti [3] — has aroused great interest in biological physics [4–8]. Despite this interest, there is still no consensus on the mechanism by which unfolded polypeptide chains with different physicochemical properties pass across the outer and inner membranes of mitochondria (OMM and IMM) [9]. One reason for this has been the lack of sufficiently precise structural and dynamic information about the translocation pores and the polypeptides to be translocated.

The mechanism of polypeptide translocation across the OMM is interesting from a physical point of view. Unlike the IMM, where a clearly defined driving force (electrochemical potential) is recognizable, the outer membrane does not have a significant transmembrane potential [10]. Thus, the source of energy for the import process is puzzling. This review delves into the protein translocation machinery within the OMM, known as translocase of the outer mitochondrial membrane (TOM). While the development of a comprehensive physical model for protein translocation in terms of transport rate and velocity remains a challenge, this article offers a tantalizing glimpse in this direction.

We commence by comparing the recent high-resolution structures of the TOM core complexes of *Saccharomyces cerevisiae* [11,12], *Neurospora crassa* [13,14] and human [15,16], as determined by electron cryo-microscopy (cryo-EM). Subsequently, we shift our focus to the present state of understanding regarding the channel dynamics observed *in vitro*. Employing cutting-edge experimental techniques involving supported droplet interface lipid bilayers and single-molecule total internal reflection fluorescence (TIRF) microscopy [17,18], recent studies have demonstrated that tethering TOM to structures adjacent to the membrane induces the closure of its two protein translocation pores [19]. In view of the fact that structural data indicate a direct interaction of TOM with the IMM

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Received: 13 February 2024
Revised: 21 March 2024
Accepted: 3 April 2024

Version of Record published: 17 April 2024

Biochemical Society Transactions (2024)
https://doi.org/10.1042/BST20231236

protein translocase TIM23 during the translocation of proteins into the mitochondrial matrix [20–23], it is tempting to infer a functional role for the *in vitro* mechanosensitivity of TOM channel activity from the nature of the functional interactions with the proteins of the inter membrane space (IMS) and IMM. In fact, we believe that a comprehensive understanding of the biomechanics of the TOM complex is required to decipher the mechanism of this process.

Protein import into mitochondria involves a panoply of key components

Over the last three decades, considerable progress has been made in understanding the biochemical background of protein translocation into mitochondria. In contrast with the bacterial ancestor, mitochondria must accommodate bidirectional protein transfer, both from the cytosol to the OMM, IMS, IMM or mitochondrial matrix, as well as protein transfer from the mitochondrial genome-encoded proteins to the IMM compartment. Whereas the mitochondrial genome encodes only a few proteins (8 in *S. cerevisiae* [24], 13 in humans [25,26] and 21 in *N. crassa* [27]), over 1000 proteins [28,29] are required for mitochondrial function, so most have to be imported from the cytosol. The TOM complex has been shown to play a central role in the recognition of most mitochondrial proteins which are nuclear-encoded [30] and serve as the main entry gate for proteins to be targeted to either the OMM, IMS, IMM or mitochondrial matrix [29,31–33]. TOM complex function is also strictly regulated [34,35]. Components located in the IMS and IMM have been proposed to exert the pulling force, facilitating translocation across the OMM [9,36,37]. Further protein import into the matrix requires an additional 'driving force', i.e. ATP hydrolysis by mtHsp70, which pulls an incoming transit protein into the matrix [38,39]. However, even if this hypothesis is correct, it still begs the question as to how a transit polypeptide can contact an IMS or IMM 'pulling protein' without it first passing unidirectionally through the TOM complex pore.

A myriad of protein components is responsible for protein transport to the different compartments. For instance, proteins destined for the IMM or the mitochondrial matrix are directed to the IMM translocases TIM22 via small TIM chaperones [40] and TIM23 [41,42], respectively, whereas mitochondrial proteins featuring characteristic cysteine motifs, which are targeted to the IMS, are received by the mitochondrial import and assembly machinery MIA [29]. The OMM-located sorting assembling machinery (SAM) orchestrates the integration of β-barrel proteins, such as the voltage-dependent anion channel (VDAC), from the IMS into the OMM [43–46]; α-helical OMM proteins are inserted into the OMM exclusively by Tom70 and the membrane-bound mitochondrial import protein complex MIM [47,48]. Export of mitochondrial genome-encoded proteins is exported from the matrix to the inner membrane by the oxidase assembly translocase OXA [49], with the TOM complex playing no direct role in this scenario.

Multiple high-resolution structures of the TOM complex support a two-pore assembly

Recent cryo-EM structures of TOM core complex isolated from *N. crassa*, human, and *S. cerevisiae* (Figure 1) [11–16,50] provide a solid basis for approaching the mechanism of protein transport across the OMM not only from a biochemical but also from a more physical point of view. In agreement with native mass spectrometry analyses [14,51] and protease accessibility mapping studies [52], previous low-resolution electron microscopy studies of *N. crassa* complexes [53,54] already indicated a dimeric subunit stoichiometry and a 19-stranded β-barrel motif for the Tom40 protein conductance channel. All high-resolution maps now consistently show two preprotein translocation pores with two β-barrels tilted at an angle of ∼20° with respect to the membrane normal, representing a common 'preprotein funnel' on the cytosolic side of the translocase (Figure 1).

In addition to the dimeric form of the TOM core complex, human and yeast TOM core complexes have also revealed a tetrameric assembly, essentially a dimer of dimers [12,15]. Chemical cross-linking studies [50,55,56] and early low-resolution structures also indicated the presence of a trimeric form with three Tom40 molecules [57,58]. However, the significance of this configuration is still unclear, as a trimeric organization is difficult to reconcile with the molecular symmetry requirements dictated by the specific protein–protein interactions in the oligomer. The trimeric form may reflect additional Tom subunits loosely associated with the TOM core complex dimer, as recently suggested by high-resolution cryo-EM of the holo complex with the peripherally associated preprotein receptor subunit Tom20 [14,16]. However, absolute certainty can only be obtained by

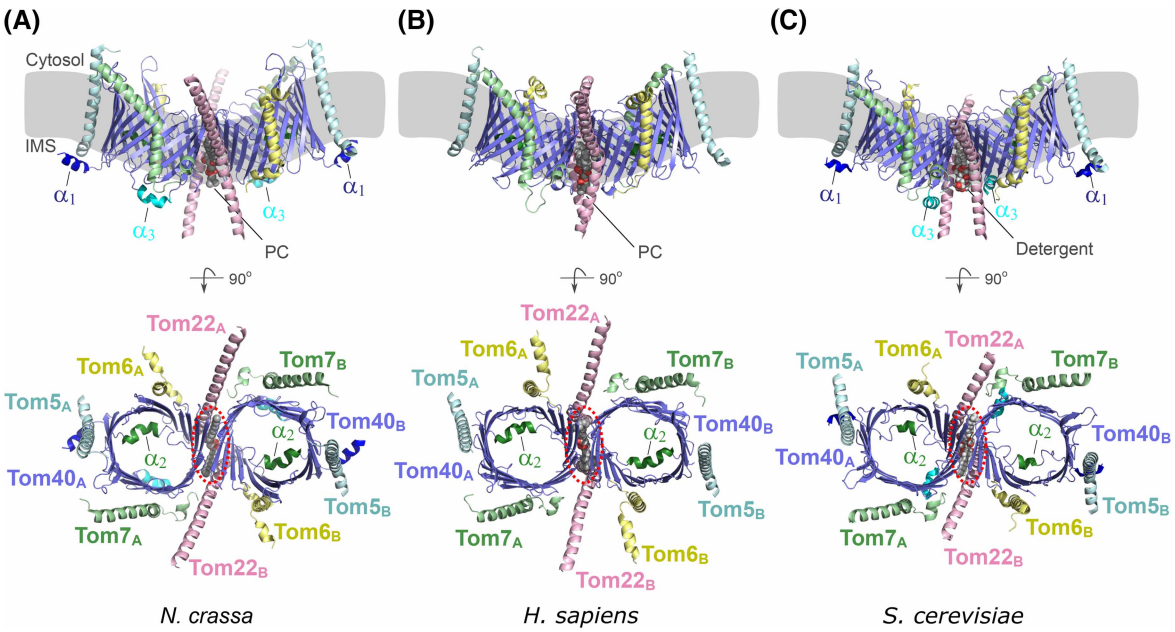

Figure 1. Cryo-EM structures of the TOM core complexes from *N. crassa*, *H. sapiens* and *S. cerevisiae*.
Atomic models of the TOM core complex of (**A**) *N. crassa* (3.3 Å resolution, PDB: 8B4I, [14]), (**B**) *H. sapiens* (2.53 Å resolution, PDB: 7VD2, [16]) and (**C**) *S. cerevisiae* (3.06 Å resolution, PDB: 6UCU, [12]). Two Tom40 β-barrels (blue) are tilted ∼20° with respect to the membrane normal. Two Tom22 transmembrane α-helices (pink) are positioned between the β-barrels and extend significantly at the mitochondrial intermembrane space (IMS) of the complex. The small subunits, Tom5 (turquoise), Tom6 (yellow) and Tom7 (green), are indicated. The lower panels show that a phospholipid ((**A**) and (**B**)) or a detergent molecule (**C**) are positioned at the IMS interface side between the two Tom22 subunits (dashed red circle).

determining the structure of the TOM holo complex, consisting of all subunits Tom70, Tom40, Tom22, Tom20, Tom7, Tom6 and Tom5, under the most native conditions possible.

Regardless of the stoichiometry of the individual subunits within the TOM core complex, it is noteworthy that a Tom40 barrel consists of 19 β-strands with a folding pattern similar to that of the mitochondrial voltage-dependent anion channel VDAC [59–62], and the division and morphology protein Mdm10 [63,64]. The odd number of β-strands is different from the porins found in Gram-negative bacteria, suggesting that the VDAC and TOM complexes share a close evolutionary relationship. Consistent with this, Tom40 dimerization occurs via β-strands β1 and β19, similar to the mechanism described for VDAC [60,65]. However, in contrast with VDAC dimers, two detergent molecules or a phospholipid run parallel to two α-helices, corresponding to the preprotein import receptor Tom22 (Figure 1), between the Tom40 β-barrels.

TOM complex shows some significant structural variation between species

Despite their overall structural similarity, all of the three TOM core complexes considered here show relatively low sequence identities. In general, the Tom40 proteins of *N. crassa* and *S. cerevisiae* show the highest sequence identity (∼35%), but both show low sequence identity with the human Tom40 (20.3% and 11.7%, respectively). For Tom5 and Tom6, even the *N. crassa* and yeast proteins show low sequence identities (25.5% and 24.6%, respectively). Surprisingly, however, Tom7 from both *Neurospora* and yeast is highly conserved (43.3%), which suggests an important but still undocumented role for this subunit. For all of the TOM core complex subunits, the *Neurospora* and yeast proteins show only weak sequence identity with the human TOM core complex subunits. Paradoxically, the low level of sequence identity between species is actually helpful, since the limited regions of complete conservation highlight their important functional role.

Comparison of the *N. crassa* TOM core complex cryo-EM structure with those of other organisms reveals that the 19-β-strand translocation pore in *N. crassa*, yeast and human TOMs, respectively, are conserved

Biochemical Society Transactions (2024)
https://doi.org/10.1042/BST20231236

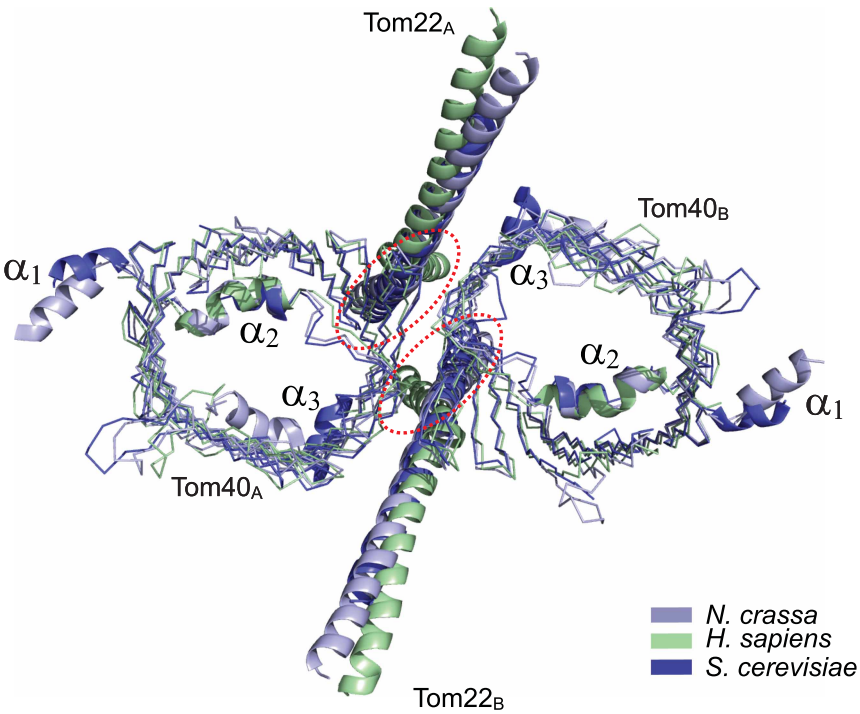

Figure 2. Structural and conformational differences between the human and fungal TOM core complexes.
The superposition of human (PDB: 7VD2), *N. crassa* (PDB: 8B4I) and yeast (PDB: 6UCU) Tom40 and Tom22 subunits is depicted, highlighting differences in α1 and α3 Tom40 helices among the species. The positions of the α1 and α3 helices of the respective complexes are shown in different colors. Tom22 is prominent on both sides of the membrane but remains partially unresolved (missing residues: *N. crassa*, 1–62 and 123–160; human, 1–62 and 119–142; yeast, 1–85 and 136–162). The position of the 'twisted' Tom22 subunits on the IMS side is indicated (dashed red circles).

(Figure 2). The *N. crassa* Tom40 structure shows two helices at the N-terminus, α1 on the IMS side of the protein, followed by α2 traversing the pore from the IMS to the cytosolic side. The C-terminus of the Tom40 includes a short α-helical segment (α3) on the IMS side. Distinctions between *N. crassa*, yeast and human Tom40 arise in helices α1 and α3. The N-terminal region of human Tom40 is anticipated to be longer and disordered, whereas it is present in the *N. crassa* and yeast models as helix α1. Similarly, the helix α3 is absent from human Tom40. Small differences between *N. crassa* and yeast Tom40 also occur in helix α3, which is rotated by 90° with respect to each other. Yeast helix α3 is longer than *N. crassa* TOM and points toward Tom22. Notably, human Tom7 features an elongated C-terminus extending toward the IMS side and an extended loop, likely taking the place of Tom40 helix α3 in the translocation exit pathway.

In all three species, Tom22 (Figure 2), although not fully resolved on both sides of the membrane, protrudes significantly from the membrane on the IMS side of the complex. The transmembrane segments of Tom22 cross the complex and bend outwards at Pro99 in *N. crassa*, Pro112 in yeast and Pro98 in human. Interestingly, human Tom22 exhibits a markedly 'twisted' conformation compared with *Neurospora* and yeast Tom22 on the IMS side of the complex (Figure 2). It remains to be seen whether this twisted conformation is a human-specific feature or one of the accessible conformations of the Tom22 subunit.

Stabilization and destabilization of the Tom40 barrel and the unusual nature of the Tom40–Tom22 dimeric interaction

There are many structural indications that the Tom40 barrel(s) are prone to conformational instability. From a purely physicochemical point of view, β-barrels with an odd number of β-stands are less stable compared with those with an even number of strands. This is due to the fact that the stabilizing hydrogen bonds between

antiparallel β-strands are able to assume a minimum energy configuration, involving 'linear' electron densities between communicating atoms, rather than those between parallel strands, which are 'bent', i.e. strained. For the Tom40 barrel, stands 1 and 19 are parallel, which would place conformational strain at this locus. Interestingly, however, the β-strands 1 and 19 are intimately involved in the interaction with Tom22. As we will elucidate below, focusing on conformational instability at this point may be of functional significance.

In general, it has been shown that thermodynamically unstable regions of β-barrel membrane channels are often stabilized by the assembly of the β-barrels into an oligomer or by the binding of extra- or intra-barrel α-helical structural protein segments or proteins [66]. Several amino acids which contribute to β-barrel thermodynamic instability have been indicated by evaluating the single-body propensity of each contributing amino acid in human [67] and *N. crassa* Tom40 [68]. Strikingly, all of the destabilizing residues appear in sequence domains, which are relatively conserved, although the destabilizing amino acids within them are not necessarily conserved (Supplementary Figure S1). This strongly indicates that for the non-conserved destabilizing residues the destabilization effect is due to a long-range effect, rather than due to specific interactions.

The nature of the phospholipid interaction with the two Tom22 subunits is particularly tantalizing. Perusal of the subunit interfaces of all cryo-EM structures shows that only part of the potential Tom40 subunit interface in the bilayer is actually involved in subunit interaction (the reduction in accessible surface area due to Tom40 dimer formation is ∼555 Å^2 (Figure 1) [16], and this is localized to about half of the Tom40–Tom22 interface at the cytosolic side. The interfacial region at the IMS is comprised almost exclusively by a Tom22–phospholipid–Tom22 interaction site (in the yeast structure the phospholipid binding site seems to be occupied by two detergent molecules) (Figure 3). In the interaction region, the aliphatic chains of the phospholipid

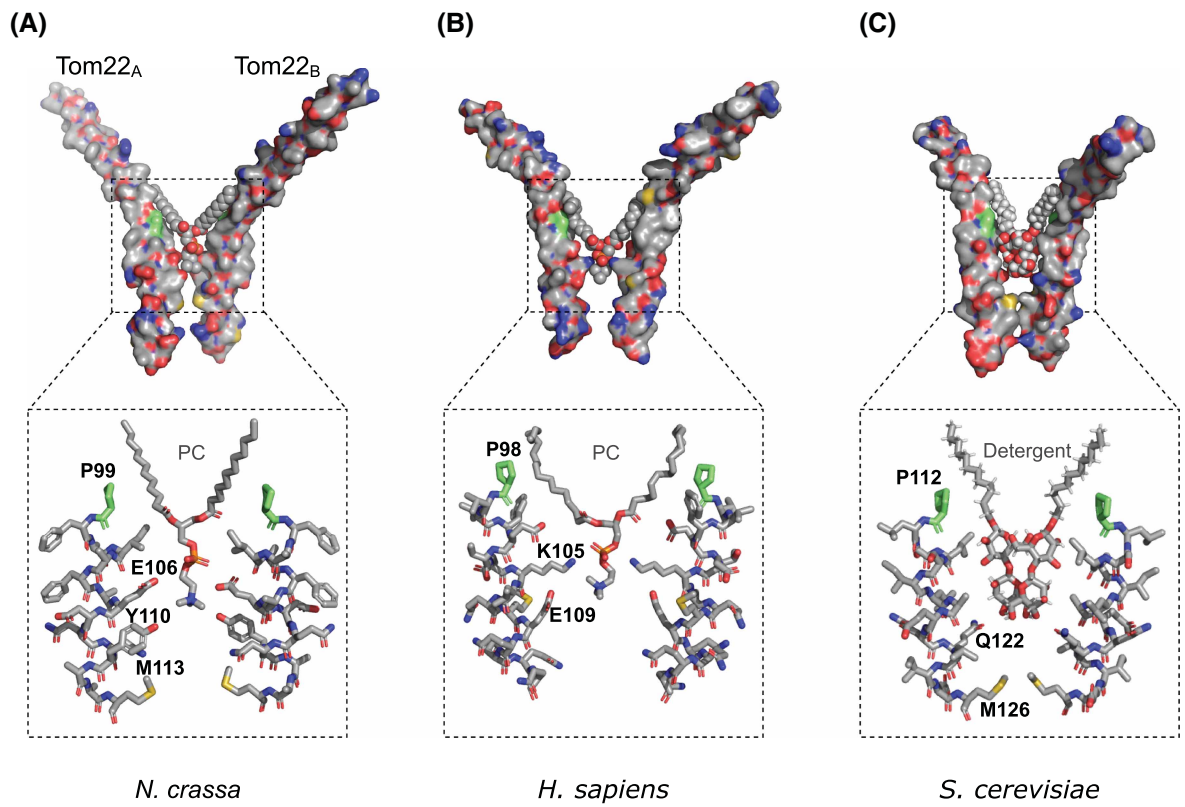

Figure 3. The phospholipid or detergent binding domain of Tom22.

Upper panels: Spacefilling representations of the two Tom22 subunits of (**A**) *N. crassa* (PDB: 8B4I), (**B**) human (PDB: 7VD2) and (**C**) yeast (PDB: 6UCU). Amino acid residues are shown in the CPK representation. TOM core complexes show an assigned phosphocholine (PC) ((**A**) and (**B**)) or *n*-dodecyl β-maltoside (**C**) between the two subunits. Lower panels: expanded views of the lipid/detergent binding sites. The lipid or detergent headgroups are held in place by electrostatic and polar interactions involving the phosphocholine moiety and nearby residues. The key residues of the binding sites are indicated.

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straddle the two Tom22 subunits, and the polar head group is sandwiched between the two Tom22 subunits such that the dipole of the (assigned) phosphocholine (PC) head group (or for the detergent, this is the polar head group of *n*-dodecyl β-maltoside) appears to be transfixed by an electrostatic field emanating from charged (but reciprocally neutralizing) amino acid residues in its vicinity (Figure 3). Notably, the electrostatic environment of the *Neurospora* and human Tom22 interaction sites (both of which bind a putative phospholipid in their structure) bear more similarity to each other, than to the yeast Tom22 interaction site, where only polar but uncharged residues participate in Tom22-detergent head group interaction. Of particular interest is that the orientation of the (assigned) PC dipole is severely distorted away from the minimum energy configuration, which in phospholipid bilayers as well as crystals in aqueous acetic acid, is oriented almost parallel to the C_2–C_3 vector of the glycerol backbone for all phospholipid head groups in membrane bilayers studied so far [69–71]. It is tempting to suspect that the strained configuration of the bound phospholipid head group is somehow contributing to the balance of conflicting forces which may be operating at the Tom22 interface, and which are crucial for their proposed contribution to reversible mechanosensitivity. Note that these arguments would also hold if the phospholipid electron density corresponded to phosphoethanolamine (PE), since biochemical studies have shown PE but not PC to be important for TOM function in *S. cerevisiae* [72]. The fact that the bound lipid can be substituted by detergent indicates that the presence of the lipid is not obligatory for structural integrity.

Evolving significance of mechanosensitive properties in shaping TOM channel dynamics

Biochemical [73], electrophysiological [74–77] and recent structural data of the TOM holo complex with the peripherally associated subunit Tom20 [14,16] suggested different structural states indicating significant dynamic conformational changes of the TOM complex. It was, therefore, surprising that mechanical interactions of the Tom22 preprotein receptors with structures in the vicinity of the membrane lead to conformational changes that result in the reversible closure of the two Tom40 pores (Figure 4) [19]. Freely diffusing TOM core complex molecules were found to 'stall' when interacting with structures adjacent to the membrane, presumably due to the local confinement of its extended polar IMS domains of Tom22. Coincident with stalling, TOM reversibly changed from an active to a weakly active and inactive channel state. The pronounced temporal correlation between lateral mobility and ion permeability implies that TOM channel gating is inherently sensitive to permanent molecular confinement and various modes of lateral mobility, including free diffusion, transiently confined diffusion or transient anchorage. To date, this observation has not been demonstrated *in vivo*. Nevertheless, it is consistent with recent studies that have identified mitochondria as mechanosensitive organelles that sense and integrate mechanical, physical and metabolic signals to adapt their morphology, network organization and metabolic functions [78–81]. It is, therefore, possible that TOM-mediated protein translocation across the OMM is also influenced by mechanical cues. Since the TOM core complex locally bends the outer mitochondrial membrane toward the IMS with an average radius of ∼140 Å [82], the anchor domains of the two Tom22 subunits should easily be able to dock to proteins in the IMS and the IMM. In light of the recent high-resolution cryo-EM structure of a TOM–TIM23 supercomplex, highlighting an interaction between Tom22 and Tim17 in the TIM23 machinery [23], it is intriguing to investigate how earlier biochemical data, indicating interactions with Tim21, Tim23 and Tim50 [22], align with this new revelation. Since one or both Tom40 channels are closed upon the capture of the TOM complex by Tom22, mechanical influences and mitochondrial dynamics might significantly affect the partitioning of mitochondrial preproteins into one of the Tom40 pores.

Integrating structural and dynamic data to explain protein translocation through the TOM complex — the paramount importance of membrane bilayer lateral pressure

The fundamental conundrum for protein translocation is that no chemical or electrochemical energy is available to drive the vectorial transport of a preprotein through the TOM complex. Thus, the forces for vectorial transport must be close to the thermal energy. In fact, the only well-known mechanism for polymer transport at the thermal energy is the so-called ratchet mechanism, whereby small localized, thermally driven forces allow small vectorial transport in an energetically heterogeneous potential field [83]. For the TOM complex, we

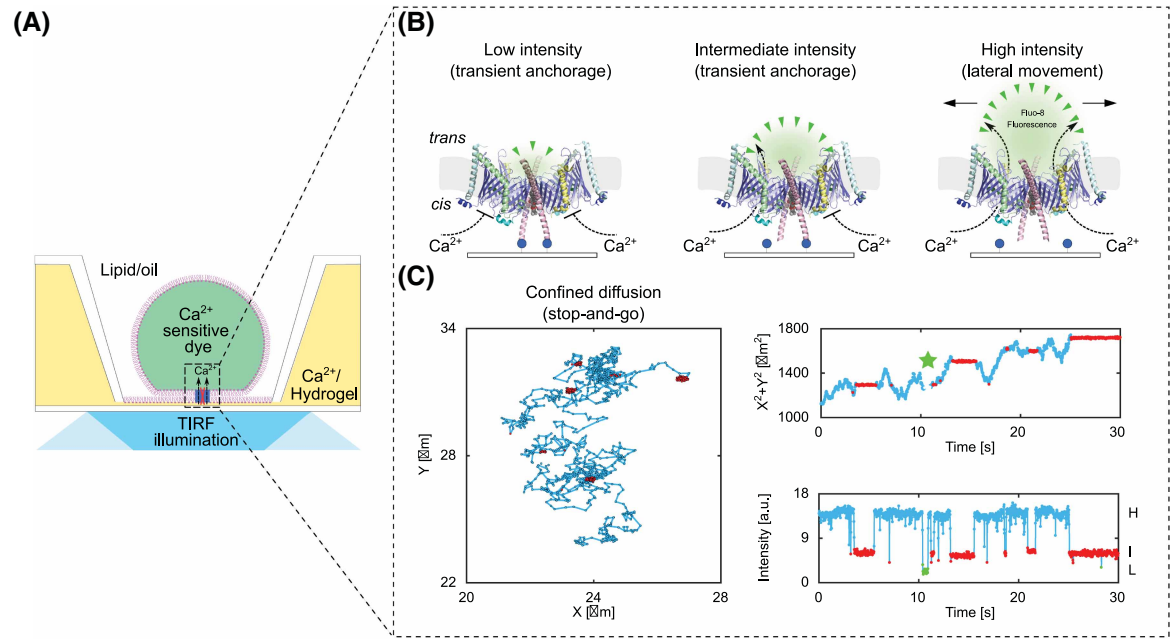

Figure 4. Correlation between lateral protein mobility and channel activity.

(**A**) Experimental TIRF setup for tracking individual TOMs and imaging their ion channel activities [18,19]. Membranes are formed by contact between aqueous droplets coated with a lipid monolayer in a lipid/oil phase and a lipid monolayer on an agarose hydrogel. The *cis* side of the membrane contains Ca^{2+} ions, while the *trans* side contains a Ca^{2+}-sensitive fluorescent dye. (**B**) Fluorescence signals reveal the local position of individual TOMs, which is used to determine their mode of lateral mobility (lateral movement vs. transient anchoring) in the membrane and the corresponding permeability states of the molecule, indicated by the level of fluorescence (low, medium and high). (**C**) Trajectories and open-closed activities of a single TOM core complex [18,19,88]. A lateral stall (red) of freely moving (blue) TOM molecules is accompanied by a partial or complete closure of a Tom40 channel. The position $(x(t)^2 + y(t)^2)$ of a channel does not change with time when the molecule is transiently stalled (red) in the membrane. Simultaneously with the arrest, the intensity of the fluorescent spot corresponding to the Ca^{2+} flux through the TOM channel changes to intermediate (I, red) or low (L, green) intensity, indicating the closure of one or two Tom40 pores, respectively.

must search for an external force which has the potential to bias the thermal energy-forces, to force the net transport of a preprotein in small steps through the pore. Here, we suggest that this external force is in fact due to the lateral pressure exerted on the TOM complex from the external surface to the center of the complex (Figure 5).

A simple calculation shows that the forces exerted on the TOM complex are within the order of magnitude necessary to be able to drive the ratchet mechanism. It is now well-established that the lateral pressure in a typical phospholipid bilayer in the liquid crystalline phase is ∼100–500 atm [84,85], where the maximum pressure P is exerted at about eight carbons of the fatty acyl chain extending into the bilayer. Using the fact that $F = P\,A$, where A is a 1 Å × 30 Å (the length of the hydrophobic phase) infinitesimal area element of the protein surface, we arrive at a value of ∼80 pN for the lateral force exerted on the protein. This is very close to the 100 pN exerted by the needle tip in atomic force microscopy [86], which is sufficient to induce protein conformational change. Since this lateral force is exerted on a TOM complex dimer, which is severely tilted with respective to the bilayer plane, but 'tethered' by a 'squashy' lipid–Tom22 interface, elementary physics dictates that the lateral bilayer force will be split into two opposing force components (Figure 5A). The ratio of these forces must be nearly equal (since the complex does not collapse) but biased to the 'downward' force due to the interaction surface in this direction. In the protein translocation event, preprotein encounters the 'funnel' of the outer surface (note that cryo-EM data do not allow the visualization of externally bound H_2O or the accumulation of ionic species, both of which may be crucial for enhancing the encounter probability [87]. We also assume that the about 20 amino acids of the unresolved the IMS domain of Tom22 (see Figure 2) do not play

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https://doi.org/10.1042/BST20231236

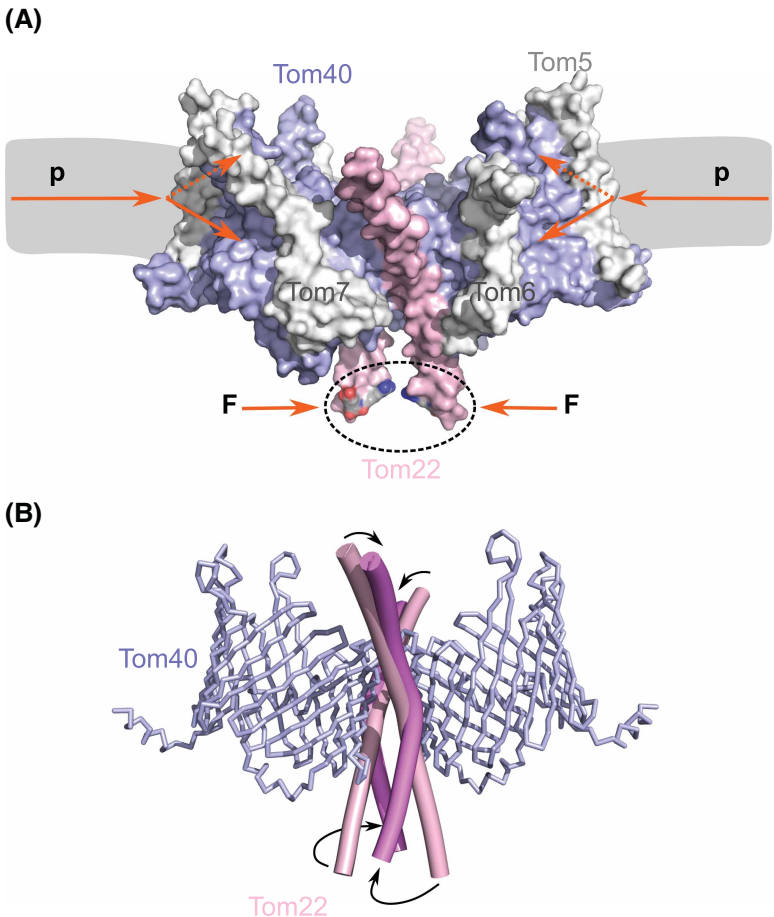

Figure 5. A hypothetical scheme for the effect of lateral pressure upon the TOM core complex.
(**A**) The figure shows a side view of the *N. crassa* TOM core complex embedded in an isopotential membrane environment (gray). The forces due to lateral pressure P are shown in red. The tilting of the Tom40 subunits localizes the dimer interaction site below the membrane plane. This directs a component of the lateral membrane exerted force to the interaction site. The second component (dashed arrow) arises from the splitting of the lateral membrane force. (**B**) The hypothetical consequences of interactions of Tom22 with structures adjacent to the membrane causing conformational changes in the IMS Tom22 segment and for the mechanism of protein translocation through the Tom40 pore are described in the main text. The movements of the two helical segments of Tom22 (pink, magenta) were implicated from the cryo-EM structures of *N. crassa* and human TOM and are indicated by arrows.

a significant role in conformational change due to high chain mobility and thus lack of electron density.). Possibly, an initial 'push' of an incoming preprotein (also due to thermal fluctuation) into the Tom40 barrel is mediated by Tom20 [14]. The Tom40 barrel, due to its low relative stability (see above), is able to distort as the polypeptide moves through the pore. The small Tom subunits 5, 6 and 7 are 'splints' which may prevent irreversible distortion of the pore and perhaps allow uniform transmission of lateral forces to the center of the TOM core complex. It is tempting to speculate that the 'squashy' phospholipid hinge (Figure 3) allows rotation of the IMS-located Tom22 domain (Figure 5B) to block the second Tom40 complex pore, as well as transiently blocking the funnel for further encounters, since the collision of two hydrophobic preproteins at the TOM complex entrance funnel may be detrimental for pore function. However, since the phospholipid hinge is principally anchored electrostatically by the zwitterionic head group, which can assume different configurations at the glycerol backbone C3 — P bond, this tilting of the Tom40–Tom22 subunit must also take place at thermal energies. The final 'pull' of the preprotein, not necessary but optimal for the ratchet mechanism, may occur via interaction with the TIM translocases [22,23]. In this mechanism, a complete reversal of the Tom40 barrel

distortion after the exit of the preprotein is mediated by the second 'upward' resultant force derived from the external lateral pressure (Figure 5).

Perspectives

- In the realm of protein translocation across biological membranes, fundamental questions persist regarding the dynamics and the driving forces behind the process. The recent high-resolution cryo-EM structures of the TOM core complexes provide an impressive starting point to elucidate which energy-dependent mechanisms predominantly govern the movement of proteins across the outer mitochondrial membrane.

- It is believed that the process of TOM-mediated recognition and translocation of proteins across the outer mitochondrial membrane operates through a Brownian ratchet mechanism, where the translocation energy is provided by molecules within the IMS and the inner mitochondrial membrane. No physical model for overcoming the free energy barrier provided by the outer membrane Tom40 pore has been presented.

- Mitochondria can sense a variety of mechanical forces due to membrane deformation, changes in lipid packing, alterations in the membrane contact sites, the cytoskeleton, or by altering the intraluminal fluid flow within organelles. Novel single-molecule techniques, together with molecular dynamics simulations and other physicochemical analyses, promise to elucidate the underlying physical principles of TOM selectivity and how the TOM complex functions *in vivo* as an unprecedented force-sensing molecule that controls the regulation of protein import into mitochondria.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
This work was funded by the German Cluster4Future initiative 'nanodiagBW' (BMBF 03ZU1208AM) (S.N.).

Author Contributions
S.N. conceived and wrote the original draft of the manuscript. R.G. and S.W. contributed substantially to the interpretations presented in the manuscript.

Acknowledgements
The authors of the review are indebted to all colleagues who contributed to the research and shared their experience in the journey toward the elucidation of the TOM structure with them, in particular Pamela Ornelas, Thomas Bausewein and Werner Kühlbrandt (Max-Planck-Institute of Biophysics, Frankfurt).

Abbreviations
cryo-EM, electron cryo-microscopy; IMM, inner membrane of mitochondria; IMS, intermembrane space; OMM, outer membrane of mitochondria; PC, phosphocholine; PE, phosphoethanolamine; SAM, sorting assembling machinery.

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