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Cryo-Electron Microscopy Structure of a Yeast Mitochondrial Preprotein Translocase

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The translocase of the outer mitochondrial membrane (TOM) complex is the main entry gate for proteins imported into mitochondria. We determined the structure of the native, unstained ~550-kDa core–Tom20 complex from *Saccharomyces cerevisiae* by cryo-electron microscopy at 18-Å resolution. The complex is triangular, measuring 145 Å on edge, and has near-3-fold symmetry. Its bulk is made up of three globular ~50-Å domains. Three elliptical pores on the c-face merge into one central ~70-Å cavity with a cage-like assembly on the opposite t-face. Nitrilotriacetic acid–gold labeling indicates that three Tom22 subunits in the TOM complex are located at the perimeter of the complex near the interface of the globular domains. We assign Tom22, which controls complex assembly, to three peripheral protrusions on the c-face, while the Tom20 subunit is tentatively assigned to the central protrusion on this surface. Based on our three-dimensional map, we propose a model of transient interactions and functional dynamics of the TOM assembly.

Keywords: electron cryo-microscopy; mitochondria; *Saccharomyces cerevisiae*; protein transport

Introduction

More than 98% of mitochondrial proteins are nuclear encoded, synthesized on cytosolic ribosomes, and imported into the organelle posttranslationally.[1,2] Two molecular machines for protein import and sorting reside in the mitochondrial outer membrane: the TOM (translocase of the outer mitochondrial membrane) complex and the SAM (sorting and assembly machinery) complex.[3] Upon passing through the TOM complex, which provides the main entry gate for protein import into mitochondria, four major pathways can be distinguished: (1) the presequence pathway for matrix proteins through the translocase of the inner membrane TIM23 complex,[4,5] (2) the carrier pathway for hydrophobic inner membrane proteins through the translocase of the inner membrane TIM22 complex,[6,7,8] (3) the sorting and assembly pathway involving the SAM complex for insertion of β-barrel proteins into the outer mitochondrial membrane,[3,9] and (4) the recently identified MIA pathway specific for import of intermembrane space (IMS) proteins.[10]

The *Saccharomyces cerevisiae* TOM complex is an assembly of seven subunits acting together at different stages of protein import: the surface receptors Tom20 and Tom70, the general import pore (GIP) consisting of Tom40,[11] Tom22, and the small Tom proteins Tom5,[12] Tom6, and Tom7. The TOM core complex consists of Tom40, Tom22, and the small Tom proteins. The core–Tom20 complex contains, in addition, the Tom20 subunit, while the TOM holo complex has all seven subunits.

Tom22, a multidomain protein, is a key component in the biogenesis, assembly, and stability of the TOM machinery.[1,3] It provides docking sites for the receptor subunits Tom20 and Tom70, which interact with the core complex.[13] Both the tail-anchored Tom22 and the signal-anchored Tom20[14] function as single-span transmembrane (TM) receptors at

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Abbreviations used: TOM, translocase of the outer mitochondrial membrane; SAM, sorting and assembly machinery; TIM, translocase of the inner membrane; IMS, intermembrane space; GIP, general import pore; EM, electron microscopy; TM, transmembrane; 3D, three-dimensional; NTA, nitrilotriacetic acid; FSC, Fourier shell correlation; BN, blue native.

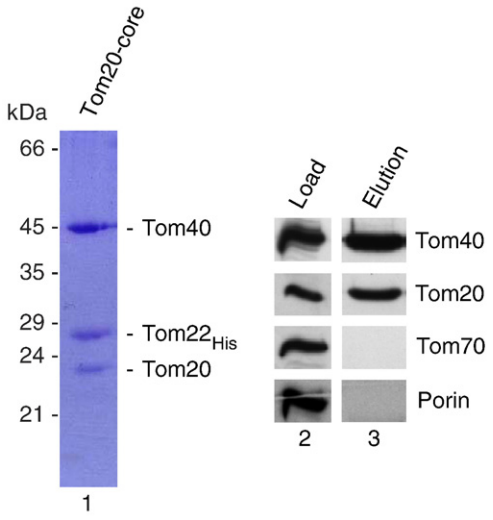

Fig. 1. Molecular composition of the Tom20–core complex. Coomassie gel of the Tom20–core complex with Tom40, $Tom22_{His}$, and Tom20 indicated. Western blot analysis confirmed the presence of Tom20, the absence of Tom70 and contaminating mitochondrial porins.

different steps of import via dynamic and multiple-mode interactions with a variety of precursors.[15] While Tom20 contains one cytosolic domain and one TM domain, Tom22 has an additional third domain exposed to the IMS. Tom40, essential for cell viability[16] and predicted to be an oligomer-forming β-barrel protein, constitutes the protein-conducting channel[17,18] and, together with the small Tom proteins,[11,12] makes up the bulk of the complex.

The oligomeric state and nature of the mitochondrial translocation channel have long been subjects of debate. Earlier electron microscopy (EM) studies of negatively stained TOM complexes in projection distinguished two populations by shape. About 15% of the particles with three apparent pores have been referred to as the holo complex in *Neurospora crassa*[19] or as the core–Tom20 complex in *S. cerevisiae*.[11] Roughly 70% of the particles in both species had only two pores and were referred to as the core complex.

Information on the three-dimensional (3D) structure of the TOM complex is essential to understand the molecular mechanisms underlying its multiple functions. The structures of the cytosolic domains of rat Tom20 in complex with a presequence peptide,[15,20] *Arabidopsis* Tom20 without ligand,[21] and *S. cerevisiae* Tom70[22] have recently been reported, but, so far, there is little information on the structure or even the precise subunit stoichiometry of the entire assembly. We now describe the structure of the core–Tom20 complex from *S. cerevisiae* as determined by cryo-EM and single-particle image processing. At 18-Å resolution, our map reveals new features of the TOM assembly. Specific labeling of the His-tagged Tom22 with nitrilotriacetic acid (NTA)–gold particles provides new insights into the location and potential role of this subunit in the core–Tom20 complex. These results provide a new platform for understanding the molecular architecture, dynamics, and assembly of the TOM complex.

Results

Cryo-EM of the TOM complex

The core–Tom20 complex (~550 kDa) was purified from digitonin-treated mitochondria by Ni–NTA affinity chromatography (Fig. 1). The isolation procedure was highly reproducible and yielded a preparation free of such contaminants as mitochondrial porins. Images of particles in a thin layer of vitreous buffer were recorded at a reduced acceleration voltage of 120 kV in order to enhance image contrast close to focus (Table 1) for particle picking, alignment, and classification. In general, the orientation of the complex with respect to the carbon support film was close to random (Fig. 3a). Triangular and rod-shaped particles, corresponding to face-on and side views of the complex, could be distinguished in the raw images. Roughly 24,000 particles were picked and processed. A 3D volume of the core–Tom20 complex was generated by random conical tilt reconstruction and projection matching

Table 1. EM and image analysis

	Negative stain	Cryo-EM	
Microscope	Philips CM120	FEI Tecnai F20	FEI Polara F30
Operating voltage (kV)	100	120	120
Dose (electrons/Å^2)	<20	<20	<20
Calibrated magnification	57,870×	60,320×	54,780×
Angstrom per pixel at specimen	3.6	3.4	3.8
Defocus range (nm)	600–2000	1200–7600	4100–13,000
Reconstruction method	RCT (44°–59° tilt)	RCT/PM	
Total no. of particles	6648	14,640	9446
Voxels[a]	9679	11,257	
Dimensions (Å^3)	123×110×83	135×135×88	
Estimated molecular mass (kDa)[b]	550	560	
Spatial resolution, FSC 5 σ threshold (FSC=0.5)	28 (33 Å)	18 Å (23 Å)	

RCT indicates random conical tilt; PM, projection matching.
[a] Based on a protein density of 0.866 Da Å^{-3}.
[b] Three-pore complex at contour levels of 3 and 5 σ, respectively.