

TIM & TOM

Mitochondrial translocases



Introduction

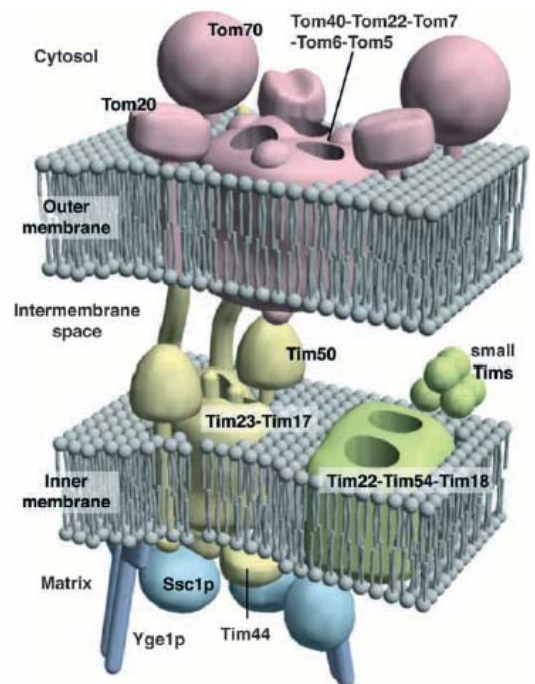
The vast majority of cellular proteins are encoded by DNA sequences in the nucleus and translated and synthesized at the ribosomes in the cytosol. For the transport into cellular organelles like mitochondria, highly complex translocation machineries exist.

The import into the different compartments of the mitochondrion (outer membrane, inner membrane, intermembrane space, matrix) is mediated by three translocases; assemblies of multiple membrane proteins. While the TOM complex with its pore forming unit Tom40 is localized in the outer membrane, TIM22 and TIM23 do their work in the inner membrane.

The translocation across the inner mitochondrial membrane is particularly accomplished by Tim23 and Tim22 as the main pore forming channel proteins. Tim23 is a ligand gated and voltage dependent cation selective channel, activated by N-terminal presequences of the mitochondrial proteins. The sequence of Tim22 shows similarities to Tim23 and it forms a hydrophilic pore when reconstituted in liposomes.

Besides their native role, the coordinated import of proteins to the designated mitochondrial compartment, a participation in processes leading to apoptosis is known for some of the members of this translocation machinery.

- Arsenic induced cell death is mediated by Tim18 in *Saccharomyces cerevisiae*.
- Overexpression of the Tim50 ortholog *ttn50* increases apoptosis in *Drosophila melanogaster*.
- The anticancer drug PK11195 is a ligand of eukaryotic TSPO (18-kDa translocator protein of the outer membrane, formerly named peripheral-type benzodiazepine receptor (PBR)) and induces apoptosis in primary chronic lymphocytic leukemia cells.



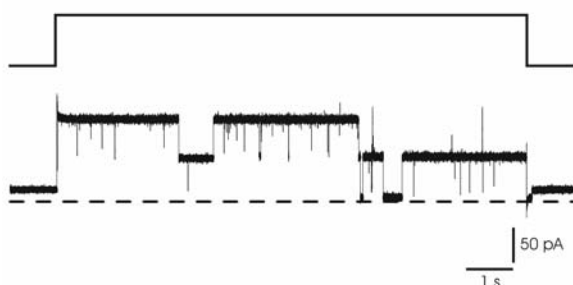
Translocator complexes in mitochondria. The TOM complex (pink) in the outer membrane, the TIM23 complex (yellow) and the TIM22 complex (light green) in the inner membrane are shown. Ssc1p and Yge1p (Mge1p), peripheral components assisting the function of TIM23 complex (blue) in the matrix, are also included.

Example: *Tom40*, a cation-selective ion channel

Tom40 is a transmembrane protein with beta-barrel structure. It is the cation selective, pore-forming component of the TOM translocase in the outer membrane of mitochondria and builds a functional channel in liposomes. When measured in planar lipid bilayers, e.g. with a **Ionovation Compact**, Tom40 showed the same main characteristics as the purified TOM complex or mitochondrial outer membrane vesicles. The single channel conductance is in the range of 360 – 900 pS, depending on the experimental conditions.

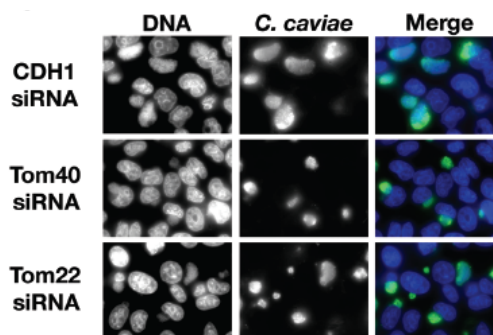
A complex voltage dependent gating behavior with several subconductance states is described for the TOM complex, which in part can be explained by two coupled channel pores (Meisinger et al., Mol Cell Biol 2001: 21, 2337-48, Künkele et al., J Biol Chem 1998: 273, 31032–39).

The presequences of mitochondrial proteins act as blockers of the ionic influx. Therefore, these pre-sequences help electrophysiologists to investigate substrate specificity with Tom40 incorporated into lipid bilayers.



Single-channel recording of isolated Tom40.

Proteoliposomes containing Tom40 were added to the cis chamber of a **Ionovation Compact** and single channels incorporated into the planar lipid bilayer were recorded at a membrane potential of +20 mV and a holding potential of 0 mV.



Tom40 and Tom22 depletion reduce *C. caviae* infection of mammalian cells

Immunofluorescence images of HeLa 229 cells transfected for 3 d with CDH1, Tom40, or Tom22 siRNA and subsequently incubated for 24 h with *C. caviae*. After fixation the cells were stained with the DNA dye Hoechst (DNA, blue) and polyclonal antibodies against *C. caviae* (*C. caviae*, green). Merge: overlay of the two images.

Although the number of infected cells was similar, the inclusions appeared smaller upon Tom40 or Tom22 depletion

Several diseases are related to Tom40:

- **Chlamydia infection**
Chlamydia *caviae* uses Tom40 and Tom22 for infection in mammalian cells. This is of particular interest, because chlamydiae are the elicitor of several severe human diseases like pneumonia, venereal diseases or arthritis.
- **Alzheimer disease**
The amyloid precursor protein forms complexes with Tom40 and Tim23, causing mitochondrial dysfunction. This may play a major role during cellular degeneration and neuronal cell death.
- **African swine fever virus (ASFV)**
ASFV infects domesticated pigs and produces acute hemorrhagic fever. Therefore, this is a potential economical threat. Tom40 is required for virus infection.