Interactions of polypeptides with the protein translocation channel of the outer membrane of mitochondria: kinetic analysis

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Almost all mitochondrial proteins are encoded in the nucleus, synthesized in the cytosol, and are posttranslationally imported into the mitochondria, which are organelles enclosed by two phospholipid membranes. The mitochondrial outer membrane protein translocase, the so-called TOM complex, plays an important role in the mitochondrial import machinery.

Most studies addressing the mechanism of protein transport through TOM complex were based on *in vitro* import studies. These studies allowed identifying numerous intermediate states along the import pathway. Quantitative analysis about the import kinetics and the mechanism of substrate interaction with TOM, however, was hampered by the unavailability of suitable protein import systems which allow monitoring substrate-TOM interactions at a single molecule level and with sufficiently high temporal resolution.

In this study, I have subjected the TOM machinery of the filamentous fungus *Neurospora crassa* to high temporal resolution electrophysiological measurements in the absence and presence of several polypeptides. A pore of the complex has a diameter of ca. 20 Å, sufficient to accommodate unfolded or partially folded proteins. At constant low voltages, channel blockage was observed in the presence of a mitochondrial presequence peptide at a single molecule level. The frequency of channel blockage progressively increased with peptide concentration and was dependent on the membrane voltage indicating an open blocker mechanism. These data provided the rate constants of substrate association and dissociation and a first glimpse into the kinetics of protein translocation through the mitochondrial TOM machinery.

Reference

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