博士学位論文の要約

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Analysis of the outer membrane insertion mechanism of yeast mitochondrial proteins

(酵母ミトコンドリアタンパク質の外膜挿入機構の解析)

Abstract:

In eukaryotic cells, mitochondria are essential not just for energy generation but also as a site for a variety of metabolic reactions, such as the synthesis of fatty acids and certain proteins. Therefore, mitochondria dysfunction leads to numerous kinds of diseases, like diabetes, Barth syndrome, Parkinson's disease, and so on.

Mitochondrial functions rely on a characteristic set of more than 1000 different proteins, most of which are encoded in nuclear DNA. After their syntheses in the cytosol, mitochondrial proteins have to be transported and sorted to a correct compartment of mitochondria, where they function correctly, such as the outer membrane (OM), intermembrane space (IMS), inner membrane (IM) and matrix. This process is carried out by the coordinated actions of the mitochondrial import and sorting machineries like TOM (translocase of the outer membrane) and TIM (translocase of the inner membrane) complexes, also smaller factors like MIM (Mitochondrial Import) complex and small TIM complex.

Presequence containing proteins that are targeted in the matrix or the IM require the TOM40 complex in the OM and the TIM23 complex in the IM for their import. Presequence-less proteins that are targeted in the IM require the TOM40 complex, the small TIMs in the IMS and the TIM23 complex for their import. Proteins that are targeted in the OM include α-helical proteins like Tom20 and Tom70, and β-barrel proteins like Tom40. The import pathway for β-barrel proteins involves the TOM40 complex, small TIMs and the Sorting and Assembly Machinery (SAM) of the OM. As for α-helical proteins, the insertion machinery is still not quite clear. Therefore, there are still unidentified pathways for the import of α-helical OM proteins.

Yeast Om45 is one of the most abundant proteins that exist in the mitochondrial OM. The N-terminal part of Om45 contains a hydrophobic segment suitable for spanning the membrane, which could serve as a mitochondrial-targeting signal. Om45 has thus been thought to be an integral OM-protein exposing its large C-terminal domain to the cytosol [1]. However, the topology of this proteins was under debate for the past few years. Therefore, the aim of my first chapter is to re-examine the localization of Om45 by biochemical approaches. In hope of establishing a novel import route for a mitochondria OM protein.

Firstly, I found Om45 exists in the IMS by protease digestion experiments. Om45 was proteinase K (PK) resistant in intact mitochondria but PK sensitive in OM raptured mitochondria. These results indicate that Om45 is associating with the OM from the IMS. *In vitro* import of N-terminally truncated or replaced mutants of Om45 showed that N-terminal 30 residues of Om45 or Tom70 are sufficient for its IMS-targeting. I then analyzed how Om45 is imported into the IMS by *in vitro* import assay using ^{35}S-labeled proteins and isolated mitochondria. My results revealed that import of Om45 into isolated mitochondria is impaired when the presequence receptor domain of Tom20 or Tom22 is deleted *in vitro*. It also competes with a matrix-targeted precursor protein in its import and an Om45 fusion protein that gets stuck on the mitochondrial surface is crosslinked to Tom40,

the channel-forming component of the TOM40 complex. More surprisingly and unlike any of the known OM proteins, Om45 import depends on both the membrane potential (ΔΨ) across the inner membrane (IM) and the TIM23 complex, the IM translocator for presequence-containing proteins. Therefore, Om45 is transported to the IMS for its insertion into the OM by a novel import pathway involving the TIM23 complex and ΔΨ.

Another interesting observation is that the C-terminal attachment of a folded domain to Om45 seems to block the Om45 pathway, so that the protein could be laterally released into the OM with the N_{in}-C_{out} topology. However, lateral opening of the β-barrel of the Tom40 channel requires simultaneous disruption of many hydrogen bonds, which is energetically unfavorable. To test this possibility, I searched for the appropriate substrate proteins. I found that Om45 pathway targeting signal fused to DHFR might be suitable because they can be released from the Tom40 channel. however, to eliminate the possibility that Om45 falls off the Tom40 channel and inserts into the OM spontaneously, I constructed fusion proteins with cytochrome b2 holding Om45 pathway targeting signal from the IMS, followed by DHFR for preventing the fusion proteins to go through the Om45 pathway. And I observed the b2 fusion proteins were inserted into the OM and left the Tom40 channel gradually. These results suggest that the Tom40 channel is opening. From *in vivo* aspect, I employed non-stop protein approach. Previously, we observed over express of non-stop Leu9 causes growth defects in dom34Δski7Δ yeast strain [2]. Since matrix targeted non-stop Leu9 stalled on ribosomes and stucked the Tom40 channel. Therefore, I used fusion proteins: the targeting signal of Om45 pathway fused to the C-terminus of non-stop Leu9 (non-stop Leu9-N). After over expressing non-stop Leu9 and non-stop Leu9-N in dom34Δski7Δ strain, I observed growth recover in the strain with expression of non-stop Leu9-N. It suggests that non-stop Leu9-N has left the Tom40 channel, so the Tom40 channel is functional for protein import again.

Taken together, my study is of great importance in getting a clearer picture of the entire import pathways of mitochondrial proteins.

[1] Uwe Ahting *et al.* (2005) *JBC.* 280, 48–53; [2] Toshiaki Izawa *et al.* (2012) *Cell Reports* 2, 447–453