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The protein import receptor MOM19 of yeast mitochondria

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We have identified the protein import receptor MOM19 of *Saccharomyces cerevisiae* mitochondria. MOM19 is exposed on the outer membrane surface and present in the mitochondrial receptor complex. Antibodies raised against MOM19 strongly inhibited the import of preproteins into isolated yeast mitochondria. Fab fragments prepared from the antibodies showed the same inhibitory effect. By using mutant mitochondria, which lacked the second import receptor MOM72, we found that the import of preproteins via MOM19 did not require the presence of MOM72. We conclude that MOM19 is required for preprotein translocation across the yeast mitochondrial outer membrane and is able to function independently of the receptor MOM72.

Mitochondrion; Receptor; MOM19; Preprotein; Protein translocation

1. INTRODUCTION

The specific import of preproteins into *Neurospora crassa* mitochondria is mediated by two receptor proteins in the outer membrane, termed MOM19 and MOM72 (mitochondrial outer membrane proteins of about 19 kDa and 72 kDa, respectively). MOM19 seems to function as main receptor for the majority of preproteins analyzed, including the preproteins with cleavable amino-terminal targeting sequences (presequences) [1,2]. MOM72 preferentially functions as receptor for preproteins with internal targeting sequences [3]. The two receptors are exposed on the surface of the mitochondrial outer membrane and apparently represent the first mitochondrial proteins that get in contact with the preproteins. The preproteins are then transferred from the receptors to the general insertion pore GIP in the outer membrane, the major component of which is MOM38/ISP42 [4,5]. The two receptors and the subunits of GIP are assembled together in a high molecular weight complex of the outer membrane, termed the mitochondrial receptor complex [6].

Much less is known about the mitochondrial protein import machineries of organisms other than *N. crassa*. In particular, only one of the import receptors has been characterized in another organism, namely MOM72 (Mas70p) in the yeast *Saccharomyces cerevisiae* [3,7]. As the putative partner of yeast MOM72, namely a hypothetical main import receptor MOM19, had not been characterized so far, the function of the import receptors in protein import into yeast mitochondria and the general role proposed for MOM19 remained questionable [8].

Recently, the mitochondrial receptor complex of yeast mitochondria was purified and found to have a similar subunit composition as the complex of *N. crassa*, leading to the identification of yeast MOM19 [9]. To analyze the function of yeast MOM19, we tried to obtain antibodies specifically directed against MOM19. For this purpose, we isolated the yeast mitochondrial receptor complex, and thus also MOM19, in chemical amounts and generated monospecific antibodies in rabbits. We show that immunoglobulins G and Fab fragments directed against yeast MOM19 strongly inhibited the import of preproteins into isolated mitochondria, supporting our proposal of a general role of MOM19 in mitochondrial protein uptake [10]. Furthermore, we analyzed the interdependence of both receptors by using a yeast mutant that selectively lacked MOM72 [3,7] and demonstrate that MOM19 functions as an independent receptor that does not require the presence of MOM72.

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Abbreviations: AAC, ADP/ATP carrier; α-MPP, α-subunit of the matrix processing peptidase; $F_1\beta$, β-subunit of the F_1-ATPase; GIP, general insertion pore; MOM19, MOM72, mitochondrial outer membrane proteins (import receptors) of about 19 kDa and 72 kDa, respectively; MOM38, mitochondrial outer membrane protein of about 38 kDa (component of GIP), identical to ISP42 (import site protein of about 42 kDa); Su9-DHFR, fusion protein between the presequence of F_0-ATPase subunit 9 and dihydrofolate reductase.

2. MATERIALS AND METHODS

The yeast mitochondrial receptor complex was purified in chemical amounts as described [9]. In brief, isolated *S. cerevisiae* mitochondria were lysed in 0.5% digitonin, and the receptor complex was purified by an affinity matrix with covalently coupled antibodies directed against yeast MOM38/ISP42. After separation by SDS-PAGE and transfer to nitrocellulose, the band corresponding to MOM19 [9] was

excised and used for generation of antibodies in rabbits. Immunoglobulins G and Fab fragments were prepared and prebound to isolated mitochondria as described [11] (papain was inhibited by iodoacetamide and antipain (Sigma)).

Published procedures [11] were used for: isolation of mitochondria from *S. cerevisiae*; synthesis of mitochondrial precursor proteins in rabbit reticulocyte lysates in the presence of [^{35}S]methionine and [^{35}S]cysteine; in vitro import of preproteins into energized mitochondria; treatment of isolated mitochondria with trypsin or proteinase K; SDS-PAGE and fluorography; immunodecoration (Western blotting) of mitochondrial proteins.

3. RESULTS AND DISCUSSION

The yeast mitochondrial receptor complex was purified in chemical amounts [9] and antibodies directed against MOM19 were generated as described in section 2. These antibodies were monospecific in reaction with isolated yeast mitochondria and the purified receptor complex (Fig. 1A). To examine if MOM19 was exposed on the outer membrane surface, isolated mitochondria were treated with trypsin, then subjected to SDS-PAGE and immunodecoration with antibodies against MOM19. Fig. 1B shows that MOM19 was degraded by trypsin via an intermediate form (MOM19′) that is about 2 kDa smaller. The protease sensitivity of yeast MOM19 is thus comparable to that of *N. crassa* MOM19 which is degraded by protease via an intermediate of about 17 kDa [1]. As controls we show the protease accessibility of MOM72, MOM38/ISP42 and the inner membrane protein ADP/ATP carrier (Fig. 1B). While the receptor MOM72 was easily degraded by protease [3], the membrane-embedded MOM38/ISP42 remained largely unaffected by the treatment with trypsin. Only at a high concentration of the protease was a fragment of MOM38/ISP42 formed. The ADP/ATP carrier was practically not degraded by the protease (Fig. 1B). Since the ADP/ATP carrier is quantitatively degraded to a smaller fragment in mitochondria with opened outer membrane [12,13], this demonstrates that the vast majority of the isolated yeast mitochondria used here possessed an intact outer membrane. We conclude that yeast MOM19 is exposed on the outer membrane surface.

We then tested the effect of antibodies directed against yeast MOM19 on the import of preproteins into isolated yeast mitochondria. The following preproteins were used: a fusion protein between the presequence of F_0-ATPase subunit 9 and dihydrofolate reductase (Su9-DHFR) [14], the α-subunit of the matrix processing

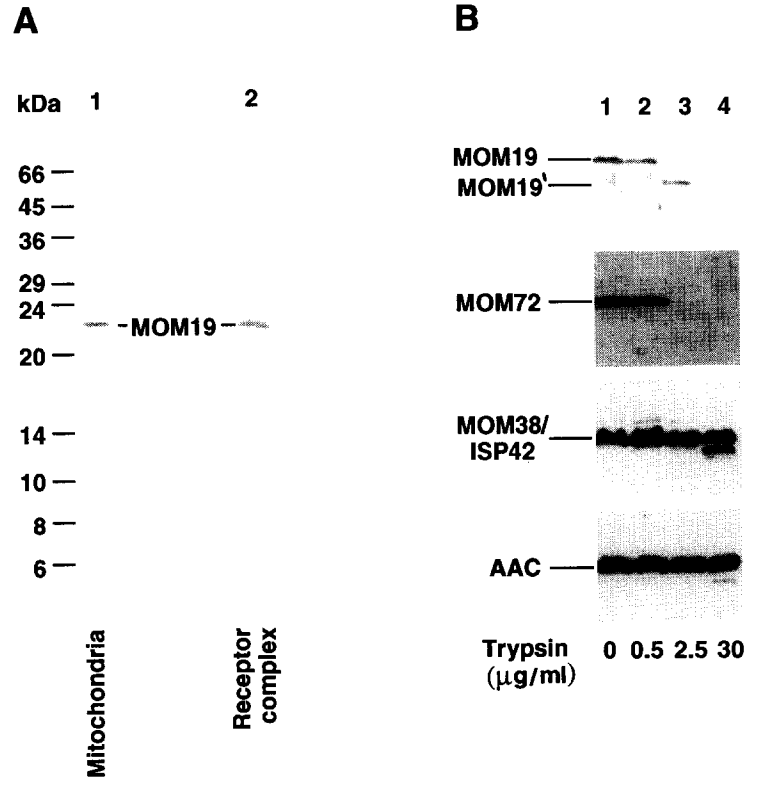

Fig. 1. Identification of yeast MOM19 by specific antibodies. (A) Immunodecoration of mitochondria and of the purified receptor complex. Isolated yeast mitochondria (50 μg protein; lane 1) or purified receptor complex (lane 2) [9] were separated by SDS-PAGE, transferred to nitrocellulose and immunodecorated with antibodies directed against yeast MOM19 (see section 2). On the gel system used, yeast MOM19 runs at 22–23 kDa [9]. (B) MOM19 is exposed on the mitochondrial surface. Yeast mitochondria (50 μg protein) were treated with trypsin and subjected to immunodecoration with antibodies directed against yeast MOM19, MOM72, MOM38/ISP42 and AAC. MOM19′, proteolytic fragment of yeast MOM19.

peptidase (α-MPP) [15,16], and the β-subunit of the F_1-ATPase ($F_1\beta$) [11]. The preproteins were synthesized in rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Upon incubation with isolated energized yeast mitochondria, the preproteins were imported and processed to the mature-sized forms (Fig. 2A, lanes 1 and 4; Fig. 2B, lane 1). The imported proteins were protected against protease added to the mitochondria (Fig. 2A, lane 4; Fig. 2B, lane 1). When the isolated mitochondria were preincubated with immunoglobulins G directed against MOM19, the import of the preproteins was strongly inhibited (Fig. 2A, lanes 2 and 5; Fig. 2B, lanes 3 and 7). Immunoglobulins G prepared from preimmune serum did not inhibit the import (Fig. 2A, lanes 3 and 6; Fig. 2B, lanes 4 and 8).

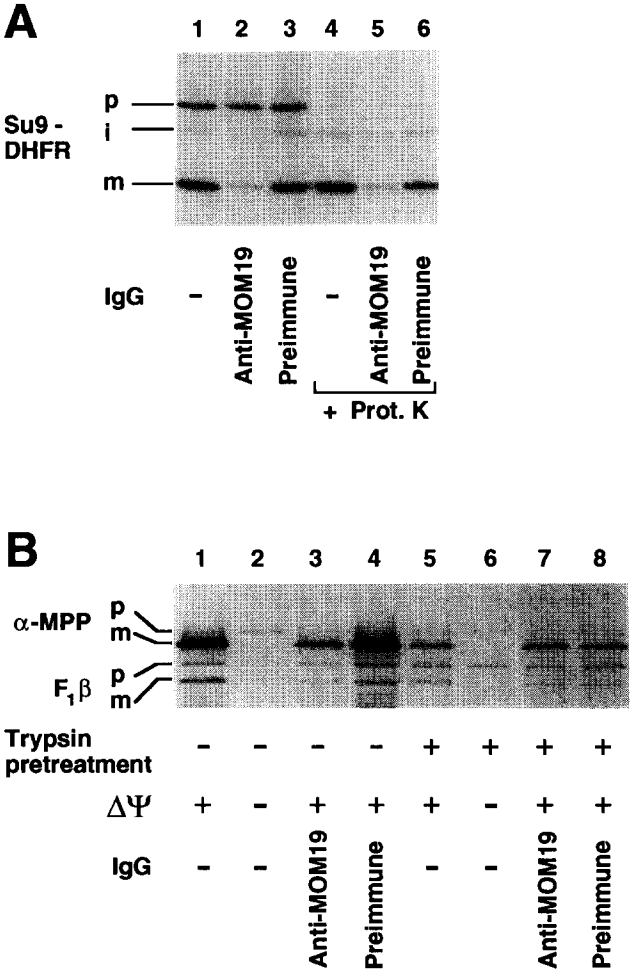

Fig. 2. Immunoglobulins G directed against MOM19 inhibit the import of preproteins into isolated yeast mitochondria. (A) Yeast mitochondria (10 μg protein) were preincubated with immunoglobulins G (IgG; 100 μg) from antiserum directed against yeast MOM19 (samples 2 and 5) or from preimmune serum (samples 3 and 6) for 35 min at 4°C (samples 1 and 3 did not receive IgGs). Reticulocyte lysate (8 μl) containing ^{35}S-labeled precursor of Su9-DHFR was incubated with the energized mitochondria for 10 min at 25°C. The samples 4–6 were then treated with proteinase K (100 μg/ml). The mitochondria were reisolated by centrifugation and analyzed by SDS-PAGE and fluorography. (B) The experiment was performed as described above with the following modifications. The mitochondria of samples 5–8 were treated with trypsin (40 μg/ml) before the incubation with IgGs. The membrane potential $\Delta\Psi$ of the mitochondria of samples 2 and 6 was dissipated by the addition of valinomycin (1 μM) (in the presence of 20 μM oligomycin) [11]. The ^{35}S-labeled precursors of α-MPP and $F_1\beta$ were imported. All mitochondria were treated with proteinase K after the import reaction. p, i, m, precursor-, intermediate-, and mature-sized forms of a protein, respectively.

To further exclude unspecific inhibitory effects of the anti-MOM19 immunoglobulins G, we made use of the so-called 'bypass import'. Mitochondria, the surface receptors of which were removed by a protease treatment, are still able to import a small amount of precursor proteins that apparently directly enter the general insertion pore GIP [17,18]. Since these protease-treated mitochondria lack MOM19, anti-MOM19 antibodies should not inhibit the bypass import. Yeast mitochondria were pretreated with trypsin, then α-MPP and $F_1\beta$ were imported. The bypass import observed required a membrane potential $\Delta\Psi$ across the inner membrane (Fig. 2B, lanes 5 and 6) as did the import into control mitochondria (Fig. 2B, lanes 1 and 2). Immunoglobulins G directed against MOM19 did not show any inhibitory effect on this residual import (Fig. 2B, lane 7). As a further support for the specific inactivation of the import receptor MOM19 by the antibodies, we demonstrate below that Fab fragments have the same inhibitory effect as immunoglobulins G.

What is the role of the import receptor MOM72 in the import of preproteins via MOM19? Of particular interest is the question if MOM19 can function as main import receptor independently of the presence of MOM72. This question can now be tackled as the availability of a yeast mutant lacking MOM72 (Fig. 3A) [3] allows the analysis of the MOM19 function alone. α-MPP and Su9-DHFR were imported into either isolated wild-type mitochondria or ΔMOM72 mitochondria. Fab fragments prepared from the anti-MOM19 immunoglobulins G strongly inhibited the import independently whether or not MOM72 was present (Fig. 3B, lanes 2 and 4). A comparable inhibitory effect was observed when immunoglobulins G were directly used (not shown). We conclude that MOM19 does not require the presence of MOM72 to mediate the import of preproteins.

We report here the functional characterization of the import receptor MOM19 of yeast mitochondria. MOM19 is exposed on the surface of the outer membrane, and antibodies against it selectively block the import of preproteins. The specificity of inactivation of MOM19 by the antibodies is supported by the following three criteria. (i) Non-specific antibodies do not block the import of preproteins. (ii) The anti-MOM19 antibodies do not inhibit the bypass import into mitochondria lacking surface receptors. (iii) Monovalent Fab fragments show the same degree of inhibition as divalent immunoglobulins G. Together with the results obtained in *N. crassa* [10], the MOM19-dependence of

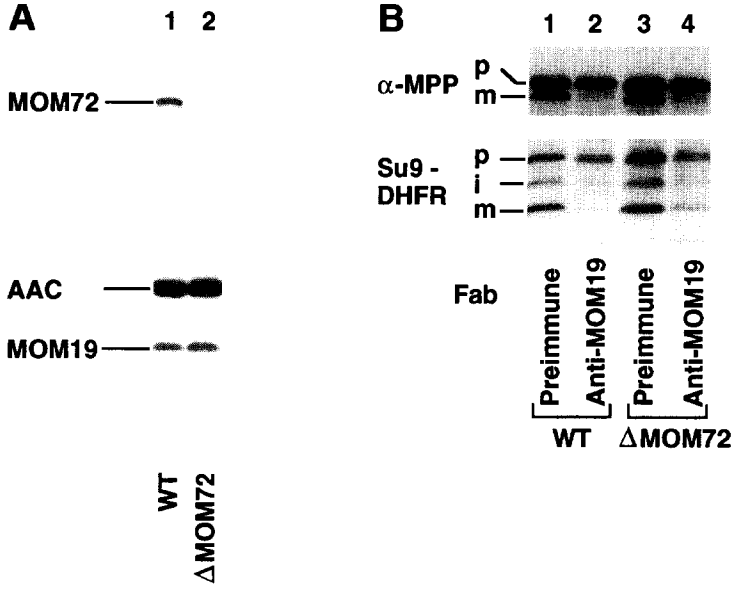

Fig. 3. Import of preproteins via MOM19 does not require the presence of MOM72. (A) Immunodecoration of wild-type yeast mitochondria and ΔMOM72 mitochondria. Mitochondria were isolated from wild-type yeast (WT; lane 1) and yeast cells lacking MOM72 (ΔMOM72; lane 2) and subjected to immunodecoration with antisera directed against MOM72, AAC and MOM19. (B) Fab fragments directed against MOM19 strongly inhibit the import of preproteins into wild-type and ΔMOM72 mitochondria. The experiment was performed as described in the legend of Fig. 2 with the following modifications. Mitochondria were isolated from wild-type or ΔMOM72 mitochondria. Fab fragments (100 μg) were used instead of immunoglobulins G.

protein import into yeast mitochondria now allows the hypothesis of a general role of MOM19 as mitochondrial import receptor. The hypothesis is underscored by the observation that a deletion of MOM72 does not affect the transport of preproteins via MOM19. MOM19 thus functions as independent main receptor of mitochondrial protein uptake. Moreover, the characterization of *S. cerevisiae* MOM19 now provides the basis for identification of its gene and a detailed genetic analysis of the structure and function of this mitochondrial import receptor.

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