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Identification of the mitochondrial receptor complex in *Saccharomyces cerevisiae*

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Mitochondrial protein import involves the recognition of preproteins by receptors and their subsequent translocation across the outer membrane. In *Neurospora crassa*, the two import receptors, MOM19 and MOM72, were found in a complex with the general insertion protein, GIP (formed by MOM7, MOM8, MOM30 and MOM38) and MOM22. We isolated a complex out of *S. cerevisiae* mitochondria consisting of MOM38/ISP42, the receptor MOM72, and five new yeast proteins, the putative equivalents of *N. crassa* MOM7, MOM8, MOM19, MOM22 and MOM30. A receptor complex isolated out of yeast cells transformed with *N. crassa* MOM19 contained the *N. crassa* master receptor in addition to the yeast proteins. This demonstrates that the yeast complex is functional, and provides strong evidence that we also have identified the yeast MOM19.

Mitochondrial outer membrane; Protein translocation; Import receptor

1. INTRODUCTION

The mitochondrial outer membrane contains machinery for the recognition and translocation of preproteins [1–5]. In mitochondria from the fungus *Neurospora crassa* two outer membrane proteins of 19 and 72 kDa, termed MOM19 and MOM72, were identified as import receptors for cytosolically synthesized preproteins [6–9]. MOM19 apparently functions as a mitochondrial master receptor [6,10,11], involved in the import of most preproteins tested including those with amino-terminal signal sequences (presequences). MOM72 mainly acts as a special receptor for a limited set of preproteins without presequences. MOM19 and MOM72 were found in a high molecular weight complex in the mitochondrial outer membrane, termed the mitochondrial receptor complex [8,12]. This complex contains five additional proteins. Four of them, MOM7, MOM8, MOM30 and MOM38, appear to be involved in the formation of the general insertion protein (pore), GIP, that is responsible for the insertion of preproteins into the outer membrane and their translocation across the membrane [13,14]. The function of the other component, MOM22, is not known.

So far, only two of the seven components of the mitochondrial receptor complex have been identified in *Saccharomyces cerevisiae* mitochondria: MOM38 (also termed import site protein of 42 kDa, ISP42) [10,15,16], and MOM72 [9,17]. In particular, the yeast equivalent of the master receptor, MOM19, has not been identified. As is the case with many yeast and *N. crassa* proteins, the sequence homologies are apparently too low to allow an identification of the homologues by hybridization or immunological crossreactions [12]. Since antibodies directed against MOM19 had been used to affinity-purify the *N. crassa* receptor complex (after lysis of the mitochondria with digitonin), the yeast receptor complex had also not been identified. This raised the question of whether the protein import machinery in the outer membrane of yeast mitochondria was functionally and structurally comparable to that of *N. crassa* mitochondria. Moreover, the identification of yeast MOM19 and other import components would be an essential prerequisite for a genetic analysis of the functions of the outer membrane import apparatus.

Here we used antibodies that specifically recognized yeast MOM38/ISP42 [10], and isolated a complex out of digitonin-lysed yeast mitochondria. The complex had a protein composition quite similar to the receptor complex of *N. crassa* and could be purified in chemical quantities. *N. crassa* MOM19 co-expressed in yeast cells efficiently assembled into the complex, demonstrating

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Abbreviations: GIP, general insertion protein/pore; MOM*y*, mitochondrial outer membrane protein of *y* kDa (apparent molecular mass in *Neurospora crassa*).

that the identified yeast complex exhibited properties expected of the mitochondrial receptor complex.

2. MATERIALS AND METHODS

Published procedures were used for the isolation of mitochondria from *S. cerevisiae* and *N. crassa*, lysis of mitochondria with digitonin-containing buffer, affinity purification of the receptor complex with antibodies coupled to protein A–Sepharose or to CNBr-activated Sepharose 4B (Pharmacia), urea–SDS-PAGE and fluorography [6,8,12,18,19]. For expression of *N. crassa* MOM19 in yeast, the coding region of MOM19 [10] was cloned into the Yep51 expression vector under the control of the galactose-inducible *GAL1* promoter.

3. RESULTS AND DISCUSSION

Saccharomyces cerevisiae cells were grown in the presence of [^{35}S]sulfate and the mitochondria isolated. The mitochondria were lysed with a buffer containing 0.5% digitonin and incubated with antibodies directed against yeast MOM38/ISP42 [10] that were coupled to protein A–Sepharose. The protein A–Sepharose with bound antibodies and proteins was harvested by centrifugation, washed, dissolved in SDS-containing sample buffer and analyzed by SDS-PAGE and fluorography. With the conditions that were routinely used for the isolation of the mitochondrial receptor complex of *N. crassa* (digitonin buffer with low salt concentration [8,12]), we were unable to reproducibly identify a putative receptor complex of yeast mitochondria. Upon inclusion of 100–300 mM NaCl in the lysis buffer, however, we observed a protein pattern of the co-immunoprecipitates that was comparable to that of the receptor complex of *N. crassa*, and thus these yeast proteins were tentatively named according to the *N. crassa* proteins (Fig. 1, lanes 1 and 2; compare Fig. 2, lane 1, and [8]). As controls we show that the bands indicated were not co-immunoprecipitated with preimmune serum (Fig. 1, lanes 3 and 4) or when the mitochondria were dissolved in SDS-containing buffer before the immunoprecipitation (Fig. 1, lane 5). The addition of salt to the digitonin buffer is obviously needed to facilitate the extraction of the receptor complex from the yeast mitochondrial outer membrane (we found that addition of salt to the lysis buffer also increased the efficiency of extraction of the *N. crassa* receptor complex). Besides the known MOM38/ISP42 and MOM72 (identified by immunoprecipitation [9]), the yeast complex contained two proteins in the range of about 20 kDa that corresponded well in size and abundance to *N. crassa* MOM19 and MOM22 (on the urea–SDS-PAGE performed here, MOM22 runs faster than MOM19). Moreover, a small protein (possibly appearing as a double band) comparable to *N. crassa* MOM8 was found. A band comparable to *N. crassa* MOM7 was not found with ^{35}S-labeled yeast mitochondria, possibly due to the lack of ^{35}S-labeled amino acid residues in the yeast MOM7 (see Fig. 2). *Neurospora crassa* MOM30 and also MOM72 are relatively loosely associated with the receptor complex [5,8,12] and are thus found in relatively small and variable amounts in the complex. The same behaviour was observed with yeast MOM72 and the putative yeast MOM30.

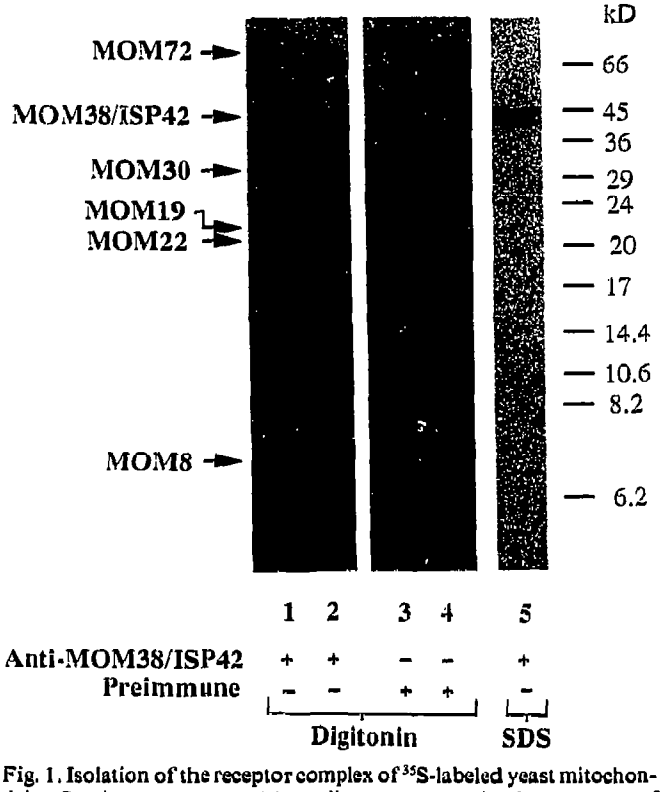

Fig. 1. Isolation of the receptor complex of ^{35}S-labeled yeast mitochondria. *Saccharomyces cerevisiae* cells were grown in the presence of [^{35}S]sulfate and the mitochondria were isolated. The mitochondria were lysed in buffer containing 0.5% digitonin as described [8,12] in the presence of 100 mM NaCl (lanes 1 and 3) or 300 mM NaCl (lanes 2 and 4). For the sample of lane 5, the mitochondria were dissolved in SDS-containing buffer and then diluted with Triton X-100-containing buffer [6]. Immunoprecipitations with antibodies directed against yeast MOM38/ISP42 (lanes 1, 2 and 5) or preimmune antibodies (lanes 3 and 4) were performed as described [6,8,10,12]. The proteins were analyzed by urea–SDS-PAGE and fluorography.

An important prerequisite for a functional analysis of the receptor complex would be the isolation of its components in chemical amounts. We covalently coupled antibodies against yeast MOM38/ISP42 to CNBr-activated Sepharose and performed the co-immunoprecipitations with yeast mitochondria that were not radiolabeled. The complexes were released from the antibodies at low pH and separated by SDS-PAGE. Fig. 2 shows the protein pattern of chemical amounts of the yeast receptor complex (lane 2) and, in parallel, the receptor complex isolated in chemical amounts from *N. crassa* mitochondria using covalently coupled antibodies against *N. crassa* MOM19 (lane 1). The protein patterns are quite comparable to each other and to the complex isolated from radiolabeled yeast mitochondria (Fig. 1, lanes 1 and 2). In addition, the protein gel shows a candidate for yeast MOM7.

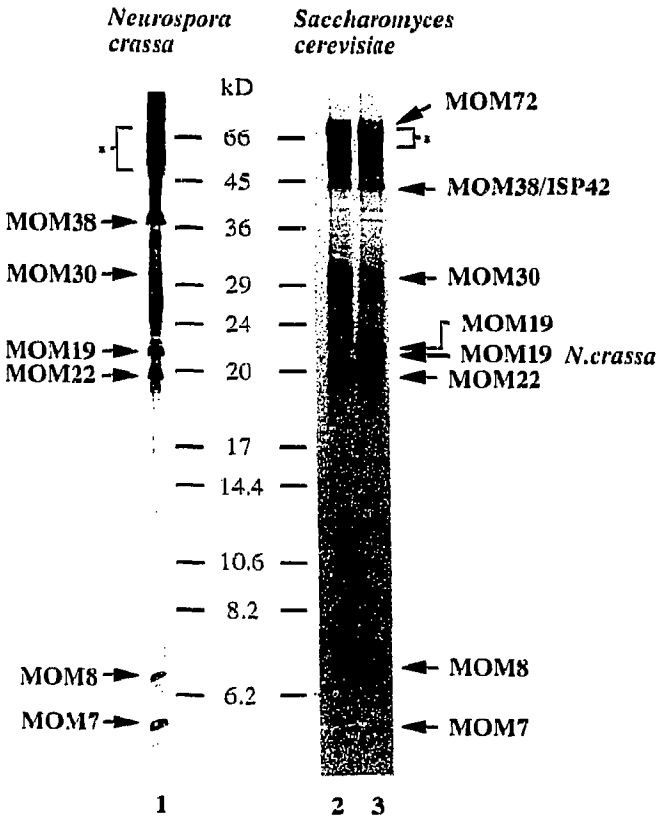

Fig. 2. Isolation of the receptor complexes of *N. crassa* and yeast mitochondria in chemical amounts. Isolated *N. crassa* (lane 1) and *S. cerevisiae* (lanes 2 and 3) mitochondria were lysed with digitonin-containing buffer in the presence of 130 mM NaCl. The yeast cells used for the mitochondria of lane 3 co-expressed *N. crassa* MOM19 (see section 2). The receptor complexes were isolated with antibodies directed against *N. crassa* MOM19 (lanes 1 and 3) or yeast MOM38/ISP42 (lane 2) that were covalently coupled to CNBr-activated Sepharose. The proteins were analyzed by urea–SDS-PAGE. The stars indicate regions of the gel with bovine serum albumin (that was present in the lysis buffer) and immunoglobulin heavy chains (that were released from the affinity matrix); these bands are present in variable amounts and are, of course, not seen when the receptor complex from radiolabeled mitochondria is analysed by fluorography (see Fig. 1 and [8,12]).

The presence of MOM38/ISP42 and MOM72 in the complex and the protein pattern similar to that of the *N. crassa* complex suggested that the yeast receptor complex had been identified. To obtain independent evidence that the yeast protein complex shown represents the mitochondrial receptor complex, we transformed yeast cells with a plasmid carrying the coding region for *N. crassa* MOM19 [10]. Mitochondria were isolated, lysed with digitonin and the immunopurification of chemical amounts of the complex was performed using antibodies directed against *N. crassa* MOM19 (this antibody does not crossreact with yeast proteins). The purified complex (Fig. 2, lane 3) contained *N. crassa* MOM19 and all the yeast components found in the co-immunoprecipitation with anti-MOM38/ISP42 antibodies. The abundance of components co-precipitated with the *N. crassa* MOM19-antibodies (lane 3) was comparable to that obtained in the co-precipitation with the yeast MOM38/ISP42-antibodies (lane 2), demonstrating that an efficient assembly of *N. crassa* MOM19 into the yeast complex had occurred. The complex thus possesses the properties expected of the mitochondrial receptor complex. We conclude that antibodies directed against yeast MOM38/ISP42 are able to co-precipitate a protein complex out of the yeast mitochondrial outer membrane that represents the receptor complex.

We report here on the identification and characterization of the mitochondrial receptor complex of yeast mitochondria. The purified complex shows a typical protein pattern comparable to that of the *N. crassa* receptor complex and thus leads to the identification of five new yeast proteins, the putative homologues of *N. crassa* MOM7, MOM8, MOM19, MOM22 and MOM30. Of particular importance is the identification of yeast MOM19. This will now open the way to a genetic analysis of the function of the putative yeast master receptor. Moreover, a genetic characterization of the GIP function will be possible. The procedure described here allows a purification of the receptor complexes of *S. cerevisiae* and *N. crassa* in chemical amounts, providing the essential basis for further biochemical analysis of the complex. Finally, the efficient assembly of *N. crassa* MOM19 into the yeast receptor complex in vivo demonstrates that the identified complex is functional. Since the *N. crassa* MOM19 is assembled into the complex while the putative yeast MOM19 is still present, this provides further evidence for the hypothesis that structure and assembly of the complex are dynamic in nature [5,12].

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