The EMBO Journal vol.15 no.6 pp.1231–1237, 1996

The Mas20p and Mas70p subunits of the protein import receptor of yeast mitochondria interact via the tetratricopeptide repeat motif in Mas20p: evidence for a single hetero-oligomeric receptor

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Protein import into yeast mitochondria is mediated by four integral outer membrane proteins which function as import receptors. These proteins (termed Mas20p, Mas22p, Mas37p and Mas70p) appear to exist as two subcomplexes: a Mas37p–Mas70p heterodimer and a less well characterized Mas20p–Mas22p complex. The subcomplexes interact functionally during protein import, but it has remained uncertain whether they are in direct contact with each other *in vivo*. Here we show that Mas20p and Mas70p can be cross-linked in intact mitochondria, or co-immunoprecipitated from digitonin-solubilized mitochondria. Furthermore, the cytosolic domains of these two proteins interact in the 'two-hybrid' system. Association of Mas20p and Mas70p is virtually abolished by a mutation in the single tetratricopeptide motif in Mas20p. This mutation specifically inhibits import of precursors that are first recognized by Mas37p–Mas70p and only then transferred to Mas20p–Mas22p. We conclude that the two receptor subcomplexes of the mitochondrial protein import receptor interact *in vivo* via their Mas20p and Mas70p subunits and that this interaction is functionally important.

Keywords: co-immunoprecipitation/cross-linking/protein translocation/*Saccharomyces cerevisiae*/two-hybrid system

Introduction

Protein import into mitochondria is mediated by several integral outer membrane proteins which function as import receptors by specifically binding mitochondrial precursor proteins (Kiebler *et al.*, 1993a; Lithgow *et al.*, 1995). Studies with *Saccharomyces cerevisiae* and *Neurospora crassa* have identified four such proteins: a 20 kDa protein termed MOM19 in *Neurospora* and Mas20p in yeast (Söllner *et al.*, 1989; Ramage *et al.*, 1993); a 70 kDa protein termed MOM72 in *Neurospora* and Mas70p in yeast (Hines *et al.*, 1990; Söllner *et al.*, 1990); a 17 kDa protein termed MOM22 in *Neurospora* and either Mas22p or Mas17p in yeast (Kiebler *et al.*, 1993b; Lithgow *et al.*, 1994; Hönlinger *et al.*, 1995; Nakai and Endo, 1995); and a 37 kDa protein termed Mas37p that has so far only been detected in yeast (Gratzer *et al.*, 1995).

Mas37p and Mas70p appear to recognize a subset of precursors that are bound to an ATP-requiring cytosolic chaperone such as mitochondrial import-stimulating factor (MSF) (Hachiya *et al.*, 1993, 1995; Gratzer *et al.*, 1995). When Mas37p and Mas70p bind the precursor–MSF complex, they accept the precursor, trigger release of MSF and transfer the bound precursor to Mas20p and Mas22p. In contrast, Mas20p and Mas22p appear to recognize mainly the basic and amphiphilic presequence of precursor proteins. This recognition appears to depend on highly acidic regions, termed 'acid bristles' in the cytosolic domains of these two proteins, and does not require that the precursor be bound to an ATP-requiring cytosolic chaperone (Haucke *et al.*, 1995; Bolliger *et al.*, 1995).

Mas37p and Mas70p can be purified from solubilized outer membranes as a stable heterodimer (Gratzer *et al.*, 1995) whereas Mas20p and Mas22p appear to form a separate subcomplex that has not yet been well defined (Lithgow *et al.*, 1995; M.Horst, unpublished data). Upon gentle solubilization of outer membranes with digitonin, a complex containing at least three of the receptor subunits can be isolated (Kiebler *et al.*, 1990), but this complex also contains additional proteins, some of them subunits of the putative protein transport channel across the outer membrane. It has thus remained open whether the two receptor subcomplexes interact directly with each other *in vivo*. Such an interaction is suggested by the fact that the cytosolic domains of Mas37p and Mas20p each contain one tetratricopeptide repeat (TPR) motif, whereas Mas70p contains seven such motifs (Goebl and Yanagida, 1991); TPR motifs are found in functionally diverse proteins and are thought to mediate protein–protein interactions (Boguski *et al.*, 1990). The TPR motifs of Mas70p seem to be functionally important, as a C-terminally truncated Mas70p lacking most of its TPR motifs is inactive as an import receptor (Riezman *et al.*, 1983).

In the present study, we have investigated whether the two subcomplexes of the yeast mitochondrial import receptor exist as a single complex *in vivo*. We show that Mas20p and Mas70p are in direct contact with each other in intact or digitonin-solubilized mitochondria and that this interaction involves the cytosolic domains of these two proteins as well as the TPR motif in Mas20p. Our results suggest that protein import into yeast mitochondria is mediated by a single hetero-oligomeric import receptor and that interaction of the two subcomplexes accelerates import of those precursors that are recognized by the Mas37–Mas70p subcomplex. Because the different subunits of the receptor recognize different features of a precursor protein, a single receptor can efficiently bind the great variety of mitochondrial precursor proteins.

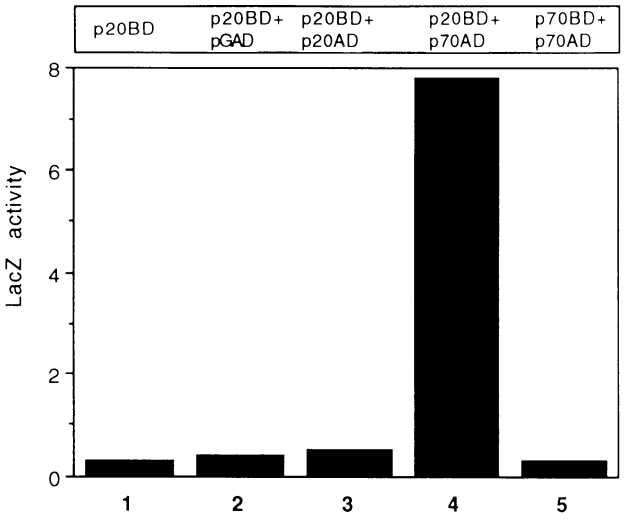

Fig. 1. The cytosolic domains of Mas20p and Mas70p interact with one another in the two-hybrid system. Yeast strain YSF526 was transformed with two different plasmids. One plasmid encoded a fusion protein between the DNA binding domain (BD) of Gal4p and the cytosolic domain of either Mas20p (p20BD; lane 1) or Mas70p (p70BD). The other plasmid encoded a fusion protein between the activation domain of Gal4p (AD) and the cytosolic domain of either Mas20p (p20AD; lane 3) or Mas70p (p70AD; lane 4). As controls, the strain was also transformed with a gene encoding only the activation domain of Gal4p (pGAD), or only wild-type Gal4p. The transformants were grown to mid-log phase in minimal medium under conditions which induce expression of the Gal4 fusion proteins, and the specific β-galactosidase (LacZ) activity of cell extracts was determined (Breeden and Nasmyth, 1985). Activity is given as a percentage of the activity measured in transformants expressing only wild-type Gal4p.

Results

Cytosolic domains of Mas20p and Mas70p interact with each other in the 'two-hybrid' system

Under most solubilization conditions, Mas70p and Mas20p do not co-fractionate, but are recovered within separate subcomplexes. In order to test whether Mas20p and Mas70p are directly associated with each other *in vivo*, we used the 'two-hybrid' system (Fields and Song, 1989). To this end, we first fused the cytosolic domains of Mas20p and Mas70p to either the DNA binding domain (BD) or the activator domain (AD) of Gal4p of yeast and then verified expression of the corresponding fusion proteins in yeast by SDS–PAGE and immunoblotting with antisera against either Mas20p or Mas70p (not shown). Finally, we measured the transcription of a *GAL1-LacZ* reporter gene in the yeast transformants by assaying β-galactosidase activity of the cell extracts (Figure 1). Co-expression of the Mas20p- and Mas70p-containing fusion proteins in the correct combination induced significant β-galactosidase activity. In contrast, co-expression of fusion proteins containing only Mas20 or Mas70p sequences, or expression of only Mas20p–BDp failed to induce the *LacZ* reporter gene. The same results were obtained with a qualitative colony filter assay (not shown).

A mutation in the TPR motif of Mas20p does not alter expression or stability of the protein

The results shown in Figure 1 suggest that the cytosolic domains of Mas20p and Mas70p can interact in intact yeast cells. How specific is this interaction? The cytosolic domain of Mas20p contains a single TPR motif, whereas the cytosolic domain of Mas70p contains seven such motifs. As TPR motifs are thought to mediate protein–protein interactions (Boguski *et al.*, 1990), we hypothesized that these motifs might be responsible for the observed interaction of Mas20p and Mas70p. To test this idea, we replaced Phe117 and Tyr118 in the TPR motif of Mas20p by alanine residues. Secondary structure predictions suggest that the resulting F117A,Y118A mutant (henceforth termed ΔTPR mutant) lacks the conserved 'knob' in the putative TPR helix and thus contains an inactive TPR motif (Figure 2A).

A haploid yeast strain expressing only ΔTPR Mas20p still grew on non-fermentable carbon sources, but the growth rate was only half that of the corresponding wild-type strain (not shown). To check whether the mutation affected expression or stability of Mas20p, we assayed mitochondria containing only wild-type or mutant Mas20p by SDS–PAGE and immunoblotting with antiserum against Mas20p, either without prior treatment, or after treatment with increasing amounts of trypsin. Both types of mitochondria contained similar levels of Mas20p, and both yielded closely similar Mas20p fragments after limited proteolysis (Figure 2B). These results suggest that the ΔTPR mutation does not drastically affect the amount or the folding of Mas20p *in vivo*. The mutation also does not significantly alter the levels of the other receptor subunits Mas22p, Mas37p and Mas70p (Figure 2C).

ΔTPR mutation greatly weakens interaction between Mas20p and Mas70p

When a digitonin extract of wild-type mitochondria was subjected to immunoprecipitation with antiserum against Mas20p, the immunoprecipitate contained not only Mas20p, but also Mas70p as well as Mas22p (Figure 3, α20; see also Kiebler *et al.*, 1990). However, the immunoprecipitate did not contain porin, an abundant outer membrane protein unrelated to the import machinery (not shown). A mock precipitation with pre-immune serum failed to precipitate either Mas20p, Mas22p or Mas70p (Figure 3, pre). When the experiment was repeated with mitochondria from the ΔTPR mutant, very little Mas70p was co-immunoprecipitated by Mas20p antiserum, whereas co-immunoprecipitation of Mas22p was as efficient as with the wild-type (Figure 3). When wild-type or mutant mitochondria were solubilized with SDS, antiserum against Mas20p immunoprecipitated only Mas20p, but not Mas70p or Mas22p (Figure 3, SDS). Thus, co-immunoprecipitation of Mas20p and Mas70p appears to reflect a specific association between these two proteins which is stabilized by the TPR motif in the cytosolic domain of Mas20p. However, the mutation does not significantly weaken the interaction of Mas20p with Mas22p.

Additional support for this conclusion was obtained by cross-linking experiments. Wild-type or mutant mitochondria were treated with the homobifunctional cross-linker dithio-*bis*-(succinimidyl propionate) (DSP), solubilized with SDS, and subjected to immunoprecipitation with antiserum against Mas20p (Figure 4) or Mas70p (not shown). Finally, the immunoprecipitates were analysed by immunoblotting for Mas70p (Figure 4) and Mas20p (not shown). Cross-linking of wild-type mitochondria generated a cross-linked product of ~90 kDa which was immuno-

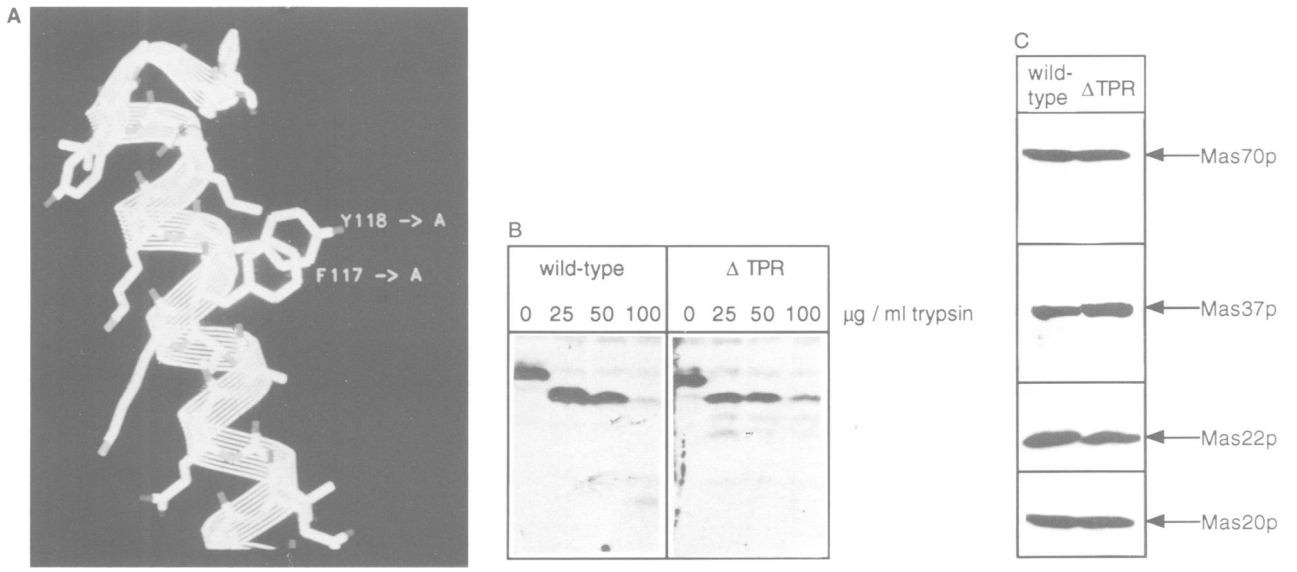

Fig. 2. The F117A,Y118A mutation in the tetratricopeptide motif of Mas20p does not denature the protein. (**A**) Ribbon diagram of the putative helical conformation of the critical region in the TPR motif of wild-type Mas20p. In the ΔTPR mutant of Mas20p, F117 and Y118 have been converted to alanine residues. The predicted 'knob'-forming residues F117 and Y118 in the wild-type helix are marked. (**B**) Trypsin treatment of intact mitochondria containing either wild-type Mas20p or the ΔTPR mutant generates very similar peptide fragments. Mitochondria (100 μg of protein in a total volume of 100 μl of isolation buffer) were treated for 30 min at 0°C with the indicated concentrations of trypsin. After adding bovine pancreatic trypsin inhibitor to 0.1 mg/ml, the mitochondria were re-isolated and analysed by SDS–PAGE and immunoblotting with antiserum monospecific for Mas20p. The blots were developed with [^{125}I]protein A and radioautography. (**C**) Mitochondria from cells expressing either wild-type Mas20p or the ΔTPR mutant of Mas20p contain similar amounts of Mas70p, Mas37p, Mas22p and Mas20p antigens. Analysis was performed on 100 μg aliquots of mitochondria essentially as described in (B).

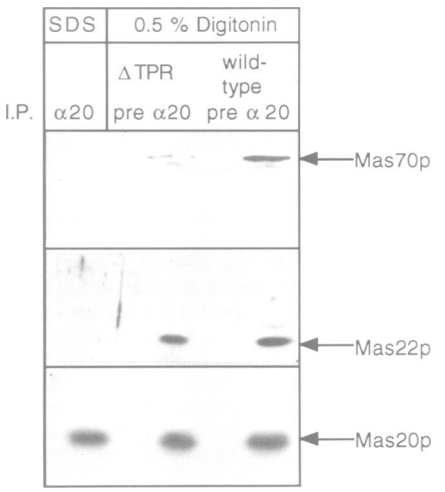

Fig. 3. Mas70p co-immunoprecipitates efficiently with wild-type Mas20p, but not with the ΔTPR mutant of Mas20p. Mitochondria (200 μg protein) containing either wild-type Mas20p or the ΔTPR mutant of Mas20p were solubilized by either 1% SDS or 0.5% (w/v) digitonin (Kiebler *et al.*, 1990). The extract was subjected to immunoprecipitation with either pre-immune serum (pre) or antiserum monospecific for Mas20p (α20) and the immunoprecipitates were analysed by SDS–PAGE and immunoblotting with antisera against Mas20p, Mas22p or Mas70p.

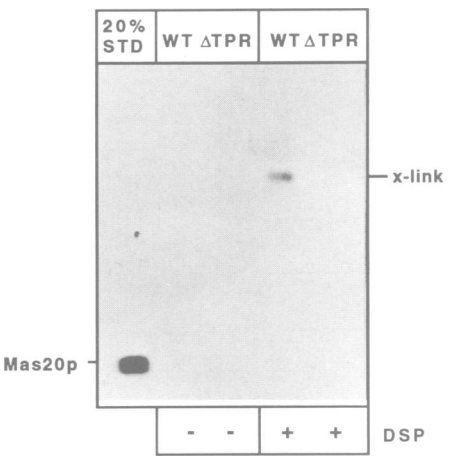

Fig. 4. Mas70p can be cross-linked to wild-type Mas20p, but not to the ΔTPR mutant of Mas20p. Mitochondria (200 μg protein) from the wild-type strain or the ΔTPR mutant were treated for 30 min at 0°C with the homobifunctional cross-linker dithio-*bis*-(succinimidyl propionate) (DSP). After quenching excess DSP with 0.2 M Tris–HCl pH 7.5, the mitochondria were isolated and solubilized for 10 min at 0°C in 1% SDS. The extract was subjected to immunoprecipitation with antiserum against Mas20p, and the immunoprecipitate was analysed by SDS–PAGE under non-reducing conditions and immunoblotting with antiserum against Mas70p. The blot was developed with [^{125}I]protein A and radioautography. 20% STD, 20% of the total amount of mitochondria used for each assay.

precipitated and immunodecorated by antiserum against either Mas20p or Mas70p. This product was not formed with mitochondria from the ΔTPR mutant (Figure 4).

ΔTPR mutation in Mas20p affects mitochondrial protein import in a similar way to a deletion or inactivation of Mas70p or Mas37p

To determine the effects of the ΔTPR mutation on the mitochondrial import of different precursor proteins, we compared the ability of wild-type and mutant mitochondria to import different precursors *in vitro* (Figure 5). We chose the following six precursors: Su9–DHFR (a fusion protein containing the first 69 amino acids of *N.crassa* F_1F_o-ATPase fused to mouse dihydrofolate reductase; Pfanner *et al.*, 1987); cytochrome b_2; COXIV–DHFR (a fusion protein containing the first 22 amino acids of the

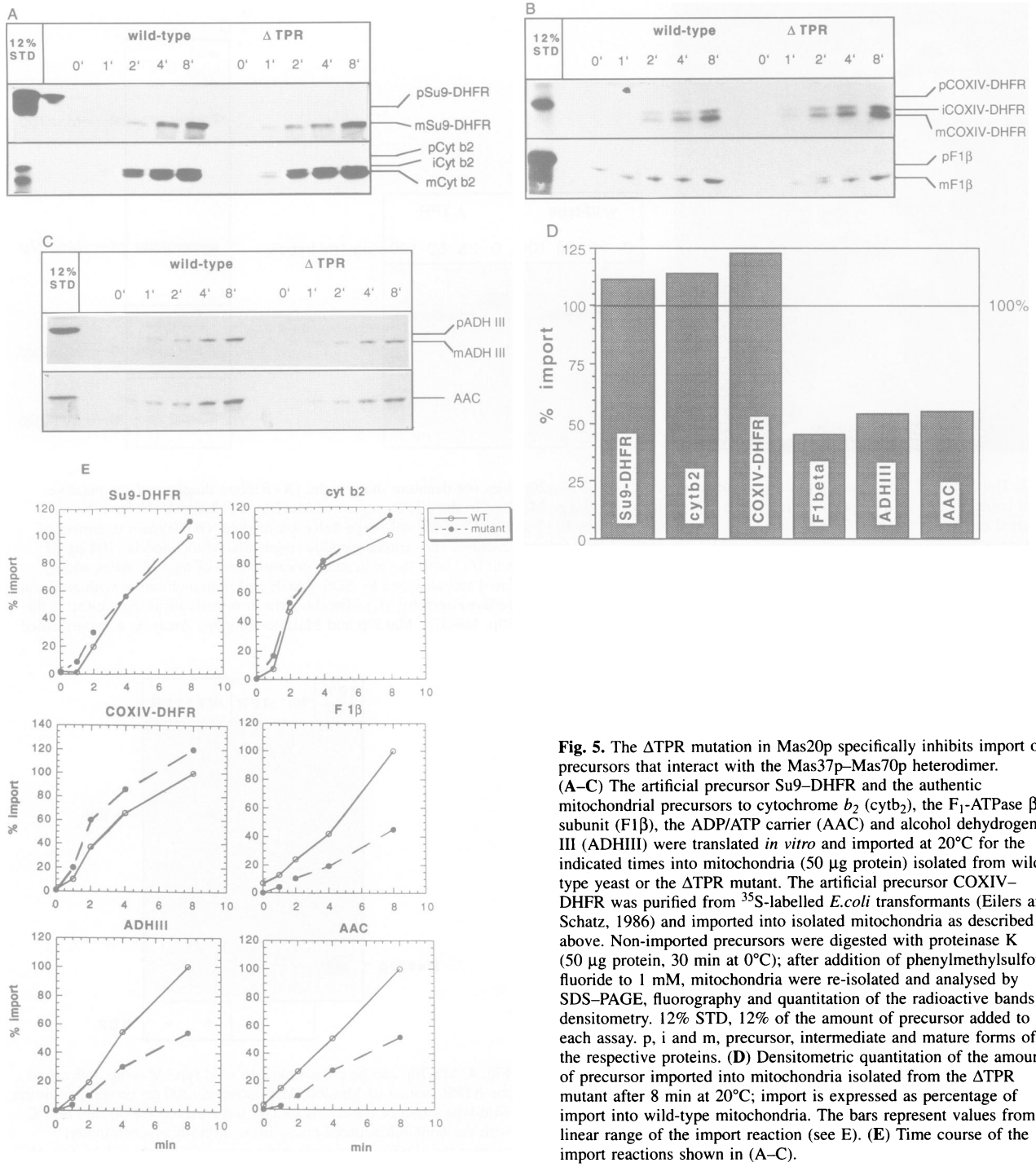

Fig. 5. The ΔTPR mutation in Mas20p specifically inhibits import of precursors that interact with the Mas37p–Mas70p heterodimer. (**A–C**) The artificial precursor Su9–DHFR and the authentic mitochondrial precursors to cytochrome b_2 ($cytb_2$), the F_1-ATPase β subunit (F1β), the ADP/ATP carrier (AAC) and alcohol dehydrogenase III (ADHIII) were translated *in vitro* and imported at 20°C for the indicated times into mitochondria (50 μg protein) isolated from wild-type yeast or the ΔTPR mutant. The artificial precursor COXIV–DHFR was purified from ^{35}S-labelled *E.coli* transformants (Eilers and Schatz, 1986) and imported into isolated mitochondria as described above. Non-imported precursors were digested with proteinase K (50 μg protein, 30 min at 0°C); after addition of phenylmethylsulfonyl fluoride to 1 mM, mitochondria were re-isolated and analysed by SDS–PAGE, fluorography and quantitation of the radioactive bands by densitometry. 12% STD, 12% of the amount of precursor added to each assay. p, i and m, precursor, intermediate and mature forms of the respective proteins. (**D**) Densitometric quantitation of the amount of precursor imported into mitochondria isolated from the ΔTPR mutant after 8 min at 20°C; import is expressed as percentage of import into wild-type mitochondria. The bars represent values from the linear range of the import reaction (see E). (**E**) Time course of the import reactions shown in (A–C).

subunit IV precursor of yeast cytochrome oxidase fused to DHFR); the F_1-ATPase β subunit; alcohol dehydrogenase III; and the ADP/ATP carrier (AAC). The first three precursors are recognized directly by Mas20p–Mas22p whereas the last three precursors are initially recognized by Mas37–Mas70p and only then transferred to Mas20p–Mas22p (Hachiya *et al.*, 1995). The precursor of COXIV–DHFR was expressed in *Escherichia coli* and purified as described (Eilers and Schatz, 1986); the other precursors were synthesized *in vitro*. All precursors were added to fully energized mitochondria under conditions in which import was linearly related to time and amount of mitochondria (Lithgow and Schatz, 1995). As shown in Figure 5, the ΔTPR mutation did not affect import of the three precursors that are directly recognized by Mas20p–Mas22p, but inhibited import of the three precursors recognized by Mas37p–Mas70p.

The import defect of the ΔTPR mutation resembles that of inactivating or deleting either Mas37p or Mas70p (Hines *et al.*, 1990; Steger *et al.*, 1990; Gratzer *et al.*, 1995). We conclude that the mutation does not inactivate the receptor function of Mas20p itself, but that it weakens the interaction of Mas20p with the Mas37p–Mas70p complex.

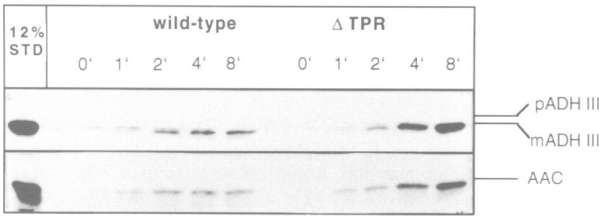

Fig. 6. Disruption of the mitochondrial outer membrane restores the import defect caused by the ΔTPR mutation in Mas20p. Mitochondria from either the wild-type strain or the ΔTPR mutant were converted to mitoplasts by selectively disrupting the outer membrane by osmotic shock (Ohba and Schatz, 1987). The mitoplasts (50 μg protein) were then assayed for the indicated times at 20°C for import of the radiolabelled precursors of the ADP/ATP carrier (AAC) or alcohol dehydrogenase III (ADHIII). The samples were analysed as described in Figure 5. p and m, precursor and mature form of each protein. The radioactive band seen at 0 and 1 min represents non-imported precursor that was not removed completely by protease treatment of the intact mitochondria.

ΔTPR mutation does not affect protein import across the mitochondrial inner membrane

In order to verify that the import defect of the ΔTPR mutant is restricted to transport across the outer membrane, we tested the effect of the mutation on protein import directly across the inner membrane. We selectively disrupted the mitochondrial outer membrane by osmotic shock, and assayed the resulting mitoplasts for import of two precursors whose import into intact mitochondria is inhibited by the mutation. The mitoplasts from both strains were equally effective in importing the two precursors across the inner membrane (Figure 6). Since bypassing the outer membrane relieves the import defect elicited by the ΔTPR mutation, this defect is specifically associated with the outer membrane.

Discussion

The problem

Earlier work has shown that mitochondrial precursor proteins bind to the mitochondrial surface by at least two different mechanisms (Wachter *et al.*, 1994; Haucke *et al.*, 1995). Precursors that are not captured in the cytosol by ATP-requiring chaperones such as MSF bind directly via their basic and amphiphilic presequence to the 'acid bristle' receptor subunits Mas20p and Mas22p. Import of these precursors is generally not affected by deleting Mas70p or Mas37p, and does not require ATP outside the mitochondria. In contrast, precursors that are captured by ATP-requiring cytosolic chaperones first bind to Mas37p–Mas70p and only then interact with Mas20p–Mas22p. Import of these precursors is inhibited by inactivation or deletion of either Mas37p or Mas70p and requires ATP outside the mitochondria.

Are these two recognition mechanisms effected by two separate, dynamically interacting receptor complexes or by a single, multifunctional receptor complex? The results of the present study favour the second possibility.

The experimental evidence

Although Mas20p, Mas22p and Mas70p can be solubilized as a complex with other components of the protein import machinery of the outer membrane (Kiebler *et al.*, 1990),

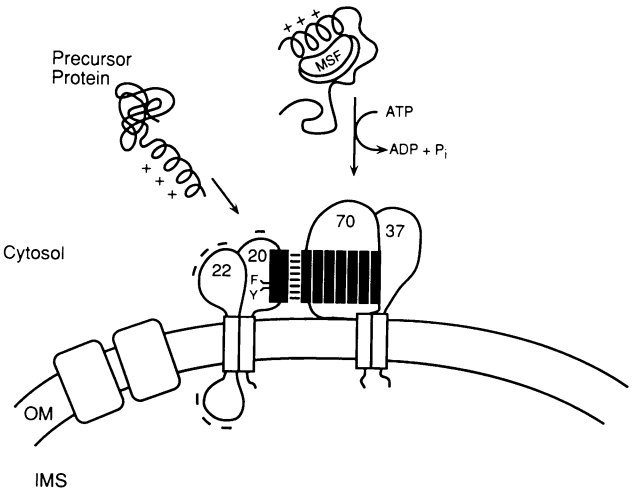

Fig. 7. Model for the interaction between the two receptor subcomplexes via the tetratricopeptide motif of Mas20p. The protein import receptor of yeast mitochondria consists of two subcomplexes which are composed of Mas20p and Mas22p, and of Mas37p and Mas70p, respectively. These two subcomplexes dissociate from each other during most solubilization conditions, but interact with one another via Mas20p and Mas70p *in vivo*. This interaction depends on the single TPR motif of Mas20p, and perhaps also on one or more of the TPR motifs of Mas70p. If the TPR motif of Mas20p is disrupted by the F117 Y118 mutation, interaction between Mas20p and Mas70p is weakened and import of precursors recognized by Mas37p–Mas70p is specifically impaired. The – and + signs signify negatively and positively charged surfaces. OM, outer membrane; IMS, intermembrane space. The rectangles with rounded corners (left) signify subunits of the protein import channel across the outer membrane.

a specific complex containing only the known receptor subunits has never been isolated. Instead, solubilization of mitochondria or mitochondrial outer membranes usually yields a stable Mas37–Mas70p heterodimer and a less well characterized complex between Mas20p and Mas22p (Gratzer *et al.*, 1995; this study; M.Horst, unpublished data). However, we now show by cross-linking, co-immunoprecipitation and the 'two-hybrid' system that the two subcomplexes interact via Mas20p and Mas70p. This interaction appears to be specific, as cross-linking and co-immunoprecipitation of the two proteins are virtually abolished by a mutation which alters only two critical residues in the single TPR motif of Mas20p. Unfortunately, we could not test the effect of this mutation on the interaction of Mas20p with Mas70p in the 'two-hybrid' system because the Gal4 fusion protein containing the mutant Mas20p domain could not be stably expressed in yeast.

We propose that the weakened interaction of the two receptor subcomplexes in the ΔTPR mutant of Mas20p selectively impairs the import of those precursors that are first recognized by Mas37p–Mas70p and only then transferred to Mas20p–Mas22p. However, it does not affect the function of Mas20p–Mas22p as a presequence receptor, and thus fails to interfere with the import of precursors that bypass Mas37p–Mas70p.

Our results make it unlikely that the ΔTPR mutation seriously affects expression or stability of Mas20p or any of the other receptor subunits *in vivo*. However, it could be argued that our methods were too insensitive

to exclude this possibility with certainty, and that the effects reported here merely reflect a functional inactivation of Mas20p. If this were true, the ΔTPR mutation should affect import in a similar fashion to a depletion or partial inactivation of Mas20p: it should inhibit import of precursors that interact directly with Mas20p–Mas22p and should have little, if any, effect on precursors that first interact with Mas37p–Mas70p. However, the mutation has exactly the opposite effect: it inhibits import of precursors that interact first with Mas37p–Mas70p and has no detectable effect on the import of the other group of precursors. Thus, we conclude that the mutation specifically impairs the interaction of Mas20p with Mas70p, and that this interaction is important for efficient import of precursors that are first recognized by Mas37p–Mas70p.

A single hetero-oligomeric protein import receptor

The results reported here and elsewhere favour the proposal (Ramage *et al.*, 1993; Lithgow *et al.*, 1995) that the multitude of mitochondrial precursor proteins are recognized by a single, multifunctional protein import receptor whose different subunits interact with different parts of a precursor protein (Figure 7). The stability of the receptor *in vivo* appears to reflect, at least in part, the interaction of TPR motifs in the cytosolic domains of Mas20p, Mas37p and Mas70p. Additional stabilization might be provided by specific docking of the transmembrane region of Mas70p to the corresponding region of the other receptor subunits (Millar and Shore, 1993, 1994). In yeast, individual subunits of this receptor complex can be deleted or inactivated without complete loss of mitochondrial protein import because the multifunctional receptor recognizes most precursors through more than one subunit. A single hetero-oligomeric receptor complex should maximize functional interaction of the different subunits and thus increase the efficiency of protein import into mitochondria.

Materials and methods

Yeast strains

Experiments were performed with the following haploid *S.cerevisiae* strains: (i) YTJB72 (*Matα ura3 his4 trp1 mas20::LEU2*) transformed with pYADE4 (Brunelli and Pall, 1993) encoding either wild-type Mas20p (wild-type) or Mas20p with a mutation in the TPR domain (ΔTPR); (ii) YSF526 (*MATα ura3 his3 ade2 lys2 trp1 leu2 gal4-542 gal80-538*) transformed with derivatives of pGBT9 and pGAD424 (Fields and Song, 1989) expressing the soluble domains of Mas20p or Mas70p as Gal4p fusion proteins.

DNA manipulations

F117 and Y118 in the TPR motif of the cytosolic domain of Mas20p were converted to two alanine residues by site-directed mutagenesis using PCR with the mutagenic primer 5′-TAC AGT CAA TGC TTT AGC CTT GCT AGC TGC TTC CAG TTC-3′. The presence of the mutation was confirmed by restriction analysis and double-stranded DNA sequencing of the entire *MAS20* open reading frame. For the experiments with the 'two-hybrid' system, DNA fragments encoding the soluble domains of Mas20p (amino acids 23–183) or Mas70p (amino acids 123–617) were amplified by PCR and subcloned into pGBT9 or pGAD424. The reading frames of the inserts were confirmed by double-stranded DNA sequencing.

Cell growth and isolation of mitochondria

Strain YTJB72 was grown on semisynthetic medium supplemented with 2% sodium lactate and 0.1% glucose, and mitochondria were isolated and purified as described (Glick and Pon, 1995). Cells derived from YSF526 were grown in minimal medium supplemented with 2% galactose, 2% ethanol, 2% glycerol and the appropriate amino acids.

Miscellaneous

Published methods were used for SDS–PAGE, standard DNA procedures, transformation of yeast and *E.coli*, total protein extracts from yeast cells, *in vitro* transcription/translation, immunoblotting, import into isolated mitochondria or mitoplasts (Ramage *et al.*, 1993; Wachter *et al.*, 1994), the generation of cross-links and immunoprecipitation (Scherer *et al.*, 1992). β-Galactosidase activity of permeabilized yeast cells was determined according to Breeden and Nasmyth (1985). Fluorograms were quantified using a computerized densitometer (Molecular Dynamics Co.). The molecular model of the TPR helix of Mas20p was generated using program 'O' (Jones *et al.*, 1991).

Acknowledgements

We thank M.Moser for the molecular modelling of the Mas20p TPR helix, M.Jäggi, L.Müller and V.Grieder for help with the artwork, H.Brütsch and T.Junne for excellent technical assistance and members of the Schatz laboratory for helpful discussions. This study was supported by grants from the Swiss National Science Foundation (31-40510.94) and the Human Capital and Mobility Program of the European Economic Community (EEC). T.L. was the recipient of a postdoctoral fellowship from the Human Frontier Science Program Organization. V.H. received a fellowship from the Carl Duisberg Foundation and is currently supported by the Boehringer Ingelheim Fund.

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Received on August 25, 1995; revised on October 30, 1995

Note added in proof

According to the new nomenclature for membrane proteins of the mitochondrial protein import machinery [Pfanner *et al.* (1996) *Trends Biochem. Sci.*, in press], the subunits of the protein import receptor are designated as follows: Tom70 (previously Mas70/MOM72), Tom37 (previously Mas37), Tom22 (previously Mas17/Mas22/MOM22) and Tom20 (previously Mas20/MOM19). Tom stands for translocase of outer membrane.