FEBS Letters 500 (2001) 12–16 FEBS 24990

An interplay between the TOM complex and porin isoforms in the yeast *Saccharomyces cerevisiae* mitochondria

Nina Antos, Malgorzata Budzińska, Hanna Kmita*

Institute of Molecular Biology and Biotechnology, Department of Bioenergetics, Adam Mickiewicz University, Fredry 10, 61-701 Poznan, Poland

Received 20 April 2001; accepted 31 May 2001

First published online 11 June 2001

Edited by Gunnar von Heijne

Abstract The outer mitochondrial membrane of *Saccharomyces cerevisiae* contains two isoforms of mitochondrial porin, known also as the voltage-dependent anion channel. The isoform termed here porin1 displays channel-forming activity enabling metabolite transport whereas the second one, termed here porin2, does not form a channel and its function is still not clear. We have shown recently that in the absence of porin1, the channel within the protein import machinery (the TOM complex) is essential for metabolite transport across the outer membrane [Kmita and Budzińska, Biochim. Biophys. Acta 1509 (2000) 6044–6050]. Here, we report that the TOM complex channel may also serve as a supplementary pathway for metabolites in the presence of porin1 when the permeability of the latter is limited and the role of the TOM complex seems to increase when porin2 is depleted.

Key words: TOM complex; Mitochondrial porin isoform; Metabolite transport

1. Introduction

The major pathway for metabolite transport across the outer mitochondrial membrane is formed by a channel called VDAC (voltage-dependent anion-selective channel), also known as mitochondrial porin [1–4]. It has been demonstrated that in one organism, different isoforms of VDAC may be present, displaying different channel-forming activities and probably playing different roles [5–9]. In the yeast *Saccharomyces cerevisiae* two VDAC isoforms have been identified [6], of which only one was proved to form a channel and be crucial to external NADH transport across the outer membrane [6,10]. This VDAC isoform, encoded by the *POR1* gene, is called VDAC1 (or porin1) and its properties are highly conserved among other species. The second yeast VDAC protein, encoded by the *POR2* gene and called VDAC2 (or porin2), probably has lost the channel-forming activity and its role in the outer membrane permeability to external NADH is rather minimal [6,10]. The oxidation of external NADH by *S. cerevisiae* mitochondria provides a simple approach for the estimation of metabolite passage through the outer membrane [10,11] since the substrate is oxidized by rotenone-insensitive and antimycin A-sensitive NADH dehydrogenase located on the outer surface of the inner membrane [12].

Since *S. cerevisiae* cells genetically depleted of the POR1 gene [6,13–15] are viable on non-fermentable carbon sources it is clear that in the absence of porin1, mitochondria are able to exchange metabolites across the outer membrane. Thus, porin1 is not the only protein supporting metabolite transport across the outer membrane of *S. cerevisiae* mitochondria. The protein(s) that could functionally substitute for porin1 is (are) also present in wild type mitochondria as there are no differences in the electrophoretic protein pattern of the outer membrane between Δ*por1* and wild type mitochondria [16,17]. So far only two channels distinct from VDAC1 have been identified in the outer membrane of *S. cerevisiae* mitochondria, namely the peptide-sensitive channel [18,19] and the TOM complex channel [20–22]. Both serve as the preprotein-conducting channels of the outer membrane [20–23] and are thought to be identical [21,22,24]. We have shown recently [25] that in Δ*por1* mitochondria of *S. cerevisiae* the TOM complex channel is involved in the permeability of the outer membrane to external NADH. This additional function of the TOM complex in the mutant mitochondria seems to be facilitated by the upregulation of its components [25,26].

Here, we report that mitochondria isolated from yeast *S. cerevisiae* cells deleted for the *POR2* gene (Δ*por2*) [6,10] also display the upregulation of the TOM complex components. In consequence, the TOM complex blockage by a given amount of a preprotein (pb_2-dihydrofolate reductase (DHFR)) is less effective when compared to that obtained with wild type mitochondria. Further, external NADH oxidation by Δ*por2* and wild type mitochondria is partially inhibited due to the TOM complex blockage, which suggests that even in the presence of porin1, the TOM complex may contribute to external NADH transport across the outer membrane of the yeast *S. cerevisiae* mitochondria.

2. Materials and methods

2.1. Yeast strains

The following *S. cerevisiae* strains were used: the parental *POR1-POR2* strain M3 (*MAT*a, *lys2 his4 trp1 ade2 leu2 ura3*), M3-2 (Δ*por2*) containing a deletion of most of the *POR2* gene, and M22-2 (Δ*por1*) containing a deletion of most of the *POR1* gene [6,10].

2.2. Isolation of mitochondria and mitoplasts

Yeast cells were grown at 28°C in YPG medium (1% yeast extract, 2% peptone, 3% glycerol) at pH 5.5 and mitochondria were isolated according to published procedures [27]. Mitoplasts were obtained by the swelling–shrinking procedure essentially as described in [27]. The

*Corresponding author. Fax: (48)-61-852 36 15.
E-mail: kmita@main.amu.edu.pl

Abbreviations: DHFR, dihydrofolate reductase; MOPS, 4-morpholinopropanesulfonic acid; TOM, translocase of the outer membrane; VDAC, voltage-dependent anion-selective channel

PII: S0014-5793(01)02575-3

estimation of the integrity of the outer membrane was based on the permeability of the membrane to exogenous cytochrome *c* [28] or on immunodecoration with antisera against marker proteins of different mitochondrial fractions.

2.3. Synthesis of pb_2-DHFR

The fusion protein called pb_2-DHFR consists of the first 167 amino acid residues of yeast cytochrome b_2 precursor comprising the mitochondrial targeting sequence with the intermembrane space sorting signal (pb_2) and the entire mouse DHFR. A radiolabelled form of pb_2-DHFR was synthesized in rabbit reticulocyte lysate (Promega) in the presence of [^{35}S]methionine. The high level expression and purification of pb_2-DHFR were performed as described in [30]. Its import competence was checked as described below.

2.4. Import of pb_2-DHFR

Mitochondria or mitoplasts were incubated in the import buffer (250 mM sucrose, 20 mM KCl, 10 mM 4-morpholinopropanesulfonic acid (MOPS)–KOH pH 7.2, 5 mM $MgCl_2$, 3% bovine serum albumin, 2 mM NADH) for 15 min at 25°C in the presence of [^{35}S]pb_2-DHFR (or 1 μg of the preprotein per 50 μg of mitochondrial protein). After washing with SM buffer (250 mM sucrose, 10 mM MOPS–KOH pH 7.2), mitochondria or mitoplasts were resuspended in the same buffer and treated with proteinase K (250 μg/ml, 10 min at 0°C), halted by the addition of 1 mM phenylmethylsulfonyl fluoride. After reisolation (10 min, 12 000 × *g*), the organelles were subjected to SDS–PAGE [29]. Import of pb_2-DHFR was visualized by fluorography (or by the ECL method following immunodecoration with anti-mouse DHFR antiserum when the preprotein was applied in chemical amounts) and quantified by ScanPack 3.0.

2.5. TOM complex blockage by chemical amounts of pb_2-DHFR

TOM complex blockage was performed as in [25] in the import buffer supplemented with 0.16 μg of valinomycin and 6 μg oligomycin per mg of mitochondrial protein in the presence of 6 μg of pb_2-DHFR per 0.1 mg of mitochondrial protein [31]. External NADH was applied at different concentrations. For accumulation of the fusion protein within both mitochondrial membranes of coupled mitochondria (the resting state condition), 2 μM of the cross-linking agent methotrexate was used as described in [32] and valinomycin and oligomycin were omitted from the incubation. To assay for pb_2-DHFR binding at the *trans* site of the TOM complex, uncoupled mitochondria or mitoplasts were washed with HS (high salt) buffer (250 mM sucrose, 10 mM MOPS–KOH pH 7.2, 120 mM KCl) [33,34]. In experiments using trypsin pretreatment, mitochondria were incubated in the presence of the protease as described in [25]. After reisolation (10 min, 12 000 × *g*), the samples were subjected to SDS–PAGE [29]. Binding of pb_2-DHFR was visualized by the ECL method following immunodecoration with anti-mouse DHFR antiserum and quantified by ScanPack 3.0.

2.6. Other methods

Protein concentrations were measured by the method of Bradford. The levels of mitochondrial proteins were visualized by the ECL method following immunodecoration with anti-yeast proper antisera. NADH oxidation was monitored spectrophotometrically (UV 1602, Shimadzu). Respiration of mitochondria and mitoplasts was monitored at 25°C with a Rank oxygen electrode in the incubation volume of 0.5 ml. Changes of the inner membrane potential ($\Delta\psi$) were monitored with a tetraphenylphosphonium-specific electrode as described in [35].

3. Results and discussion

3.1. In the absence of any mitochondrial porin isoform, S. cerevisiae mitochondria display the upregulation of the TOM complex components

We have shown recently [25] that porin1-depleted mitochondria of *S. cerevisiae* have profoundly increased levels of the TOM complex components, namely Tom40 (the main constituent of the TOM complex channel) and Tom70 (one of the preprotein receptors). We have concluded that the upregulation of the Tom proteins probably facilitates the observed involvement of the TOM complex in metabolite transport across the outer membrane. Since it has been reported that porin2 probably does not form a permeability pathway across the outer membrane of *S. cerevisiae* mitochondria [10], we expected that the levels of Tom40 and Tom70 in porin2-depleted mitochondria should be comparable to those observed with wild type mitochondria. However, as shown in Fig. 1A,B, in Δ*por2* mitochondria both proteins were present in higher amounts that in wild type mitochondria although the amounts were smaller than in mitochondria of the Δ*por1* mutant derived from the same parental strain [6,10] and used here as a control. Moreover, for both mutant mitochondria the upregulation was more pronounced in the case of Tom70. The upregulation of Tom70 observed for the applied Δ*por1*

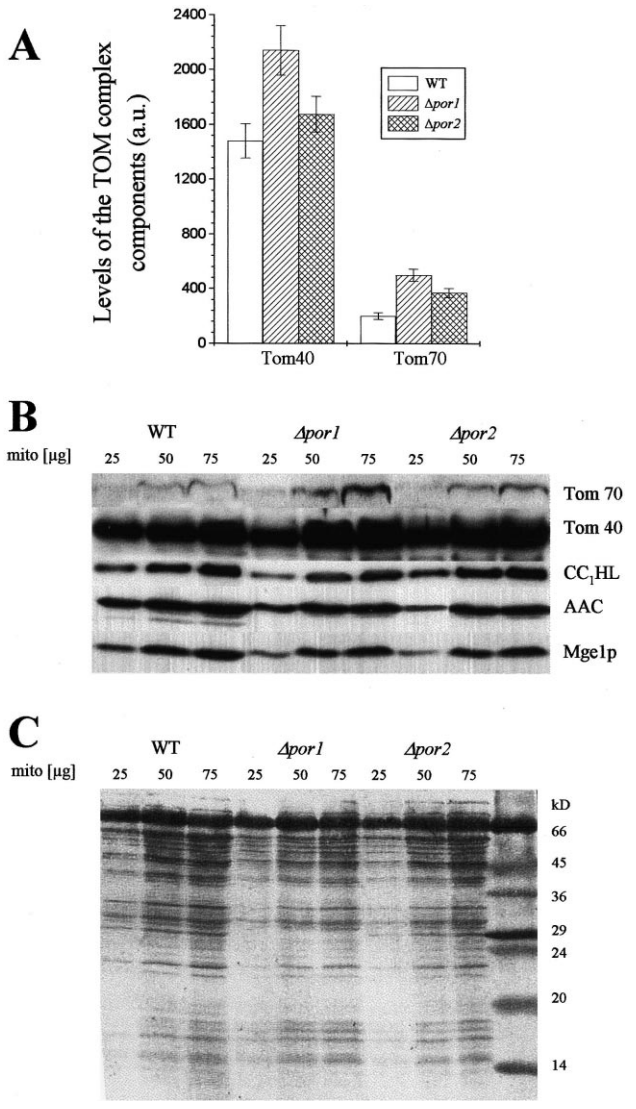

Fig. 1. The levels of the TOM complex components represented by Tom40 and Tom70 in wild type, Δ*por1* and Δ*por2* mitochondria of *S. cerevisiae*. A: Quantitative analysis of the Western blot results, obtained with 50 μg of the studied mitochondria, by ScanPack 3.0. Data are mean values ± S.E.M. of eight independent experiments. B: Typical results of Western blot of the studied mitochondria with anti-yeast mitochondrial protein antisera. C: Loading control of results shown in B. Prior to immunodecoration proteins were transferred to nitrocellulose membrane using the wet blotting technique and stained with Ponceau S.

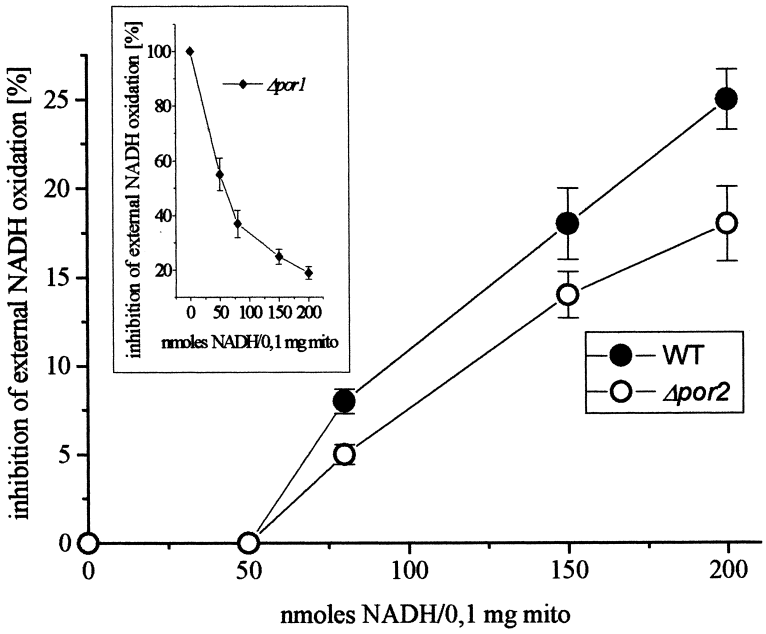

Fig. 2. Inhibition of external NADH oxidation by wild type and Δ*por2* mitochondria imposed by TOM complex blockage by pb_2-DHFR. Mitochondria were incubated under *trans* site binding conditions in the presence of 6 μg of pb_2-DHFR per 0.1 mg of mitochondrial protein as described in Section 2. Different concentrations of external NADH were applied and their oxidation was recorded spectrophotometrically. The inhibition shown in the inset was obtained under the same conditions with mitochondria of the Δ*por1* mutant derived from the applied parental strain. The levels of inhibition varied by not more than 14% in various experiments.

mitochondria was clearly stronger than that previously reported for another porin1-depleted mutant [25]. This discrepancy might be explained by strain-specific phenotypes observed after disruptions of some yeast genes, e.g. *TIM18* [36]. To test that the upregulation of the TOM complex components does not result from incorrect determination of protein concentrations, immunodecoration of different non-Tom proteins was performed with titration of different protein amounts of mutant and wild type mitochondria. As shown in Fig. 1B, none of chosen control proteins, i.e. CC_1HL (cytochrome c_1 heme lyase), AAC (ADP/ATP carrier) and Mge1p (a component of the mitochondrial Hsp70 machinery), were upregulated in Δ*por1* and Δ*por2* mitochondria. Fig. 1C serves as a loading control for results shown in Fig. 1B. Summing up, the deleted porin isoform is decisive for the extent of upregulation of the TOM complex components observed with yeast mutant mitochondria derived from the same parental strain. While the role of the upregulation has already been explained, at least partially, in the case of Δ*por1* mitochondria [25], it is unclear in Δ*por2* mitochondria. Thus, the question arises whether the increased levels of the TOM complex components could contribute to functions of Δ*por2* mitochondria also due to the channel-forming activity of the complex.

3.2. *The blockage of the TOM complex by pb_2-DHFR causes different modes of inhibition of external NADH oxidation, dependent on the depleted porin isoform.*

We next investigated whether the upregulation of the TOM complex components observed with Δ*por2* mitochondria might contribute to the permeability of the outer membrane to metabolites as was reported for Δ*por1* mitochondria [25]. For that purpose, we compared the influence of TOM complex blockage on external NADH oxidation by wild type and Δ*por2* mitochondria. We have shown recently [25] that a fusion protein termed pb_2-DHFR incubated with *S. cerevisiae* mitochondria under *trans* site binding conditions [33,34,37] blocks the protein import channel within the TOM complex. Since we found that the levels of the upregulation of the TOM complex components connected with *POR1* gene disruption could depend on strain background we applied mitochondria of the Δ*por1* mutant derived from the applied parental strain as an additional control. As shown in Fig. 2, the blockage of the TOM complex caused different modes of inhibition of external NADH oxidation, depending on the type of studied mitochondria. In the case of the applied Δ*por1* mitochondria (inset) the inhibition decreased with increasing concentrations of external NADH as we have shown recently for another porin1-depleted mutant [25], which suggests that external NADH using the TOM complex channel to cross the outer membrane prevents pb_2-DHFR from binding at the *trans* site of the complex. However, a higher amount of pb_2-DHFR had to be used in the present study to obtained comparable levels of inhibition in the presence of a given external NADH concentration, which might result from different levels of the TOM complex components in mitochondria of Δ*por1* mutants. When the same amount of pb_2-DHFR was incubated with wild type and Δ*por2* mitochondria the inhibition of external NADH oxidation appeared at its concentration of approximately 50 nmol per 0.1 mg of mitochondrial protein and increased in the presence of higher concentrations of the substrate (see also [26]). The inhibitory effect of pb_2-DHFR at a given concentration of external NADH was weaker with Δ*por2* mitochondria than with wild type ones, which might result from the upregulation of the TOM complex components in the mutant mitochondria. Similar to Δ*por1* mitochondria [25], the inhibitory effect of pb_2-DHFR observed with Δ*por2* and wild type mitochondria disappeared in mitoplasts, i.e. after the removal of the outer membrane. The effect was

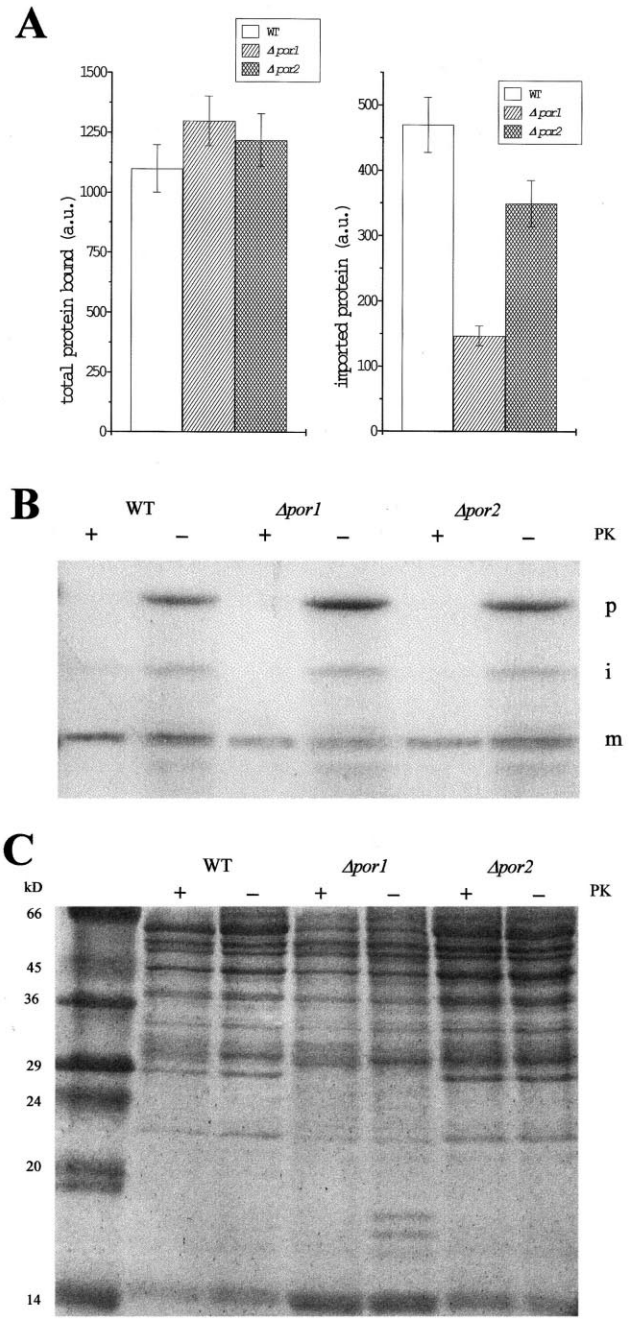

Fig. 3. Efficiency of pb_2-DHFR import into mitochondria of wild type, Δ*por1* and Δ*por2* *S. cerevisiae* strains. A: The levels of [^{35}S]pb_2-DHFR binding and its import into wild type and mutant mitochondria. Bands representing different forms of [^{35}S]pb_2-DHFR were visualized by fluorography and quantified by ScanPack 3.0. The obtained data varied by not more than 10% in various experiments. B: Typical results of [^{35}S]pb_2-DHFR import into wild type and mutant mitochondria. C: Loading control of results shown in B. Prior to fluorography the gel was stained by Coomassie brilliant blue G250.

also remarkably decreased by trypsin pretreatment of mitochondria causing the removal of the surface receptor of the TOM complex (not shown). Since in the absence of the receptors preprotein binding to the *trans* site is significantly slowed down [33], the data point to involvement of the TOM complex in external NADH transport across the outer membrane in all types of studied mitochondria. It might be concluded that in Δ*por1* mitochondria the TOM complex channel supports metabolite transport incessantly whereas in wild type and Δ*por2* mitochondria the additional function of the channel is triggered under conditions of insufficient permeability of porin1.

It has been shown that porin1 restricts the traffic of external NADH across the outer membrane of the yeast *S. cerevisiae* mitochondria under conditions leading to faster transport of the substrate through the membrane, i.e. in the uncoupled state [38]. Therefore the permeability of porin1 could be regarded as a limiting step in transport of higher concentrations of external NADH oxidized quickly by wild type and Δ*por2* mitochondria under *trans* site binding conditions. Thus, under these conditions, some amounts of the substrate might be transported across the outer membrane by the TOM complex channel. The conclusion seems to be supported by the lack of inhibition of external NADH oxidation in the case of wild type and Δ*por2* mitochondria incubated with pb_2-DHFR in the presence of methotrexate in resting state conditions. The latter results in a slower rate of external NADH transport into mitochondria than the *trans* site binding conditions. On the other hand, methotrexate stabilizing the folded state of DHFR prevents the part of pb_2-DHFR from translocation through the mitochondrial membranes. This causes pb_2-DHFR to link only a part of the TOM complex pool to the protein import machinery of the inner membrane (the TIM complex) as the TOM complex is present in large excess over the TIM complex [31,39]. Therefore under the resting state conditions applied in the presence of methotrexate, transport of some amounts of external NADH might be supported by the TOM complex and not influenced by the presence of pb_2-DHFR.

3.3. Deletion of porin1 or porin2 weakens pb_2-DHFR import into *S. cerevisiae* mitochondria but not into mitoplasts

To check whether the upregulation of the TOM complex components may result in a deeper involvement of the complex in transport of external NADH under resting state conditions, we studied import of the radiolabelled form of pb_2-DHFR into wild type, Δ*por1* and Δ*por2* mitochondria. In this case, import of the preprotein into the mutant mitochondria should be weakened in the presence of external NADH when compared to results obtained with wild type mitochondria. It should be emphasized here that in the presence of the chosen external NADH concentration (200 nmol per 0.1 mg of mitochondrial protein), the effect of pb_2-DHFR bound at the *trans* site of the TOM complex was comparable for all studied types of mitochondria (see Fig. 2). The import reaction was performed simultaneously for all types of mitochondria in the presence of the same amount of [^{35}S]pb_2-DHFR. The total amount of [^{35}S]pb_2-DHFR associated with each type of mitochondria (Fig. 3A,B) was determined by quantification and addition of bands representing the precursor (p), intermediate (i) and mature (m) forms of the preprotein obtained in the absence of proteinase K (−PK). The amounts of imported protein (Fig. 3A,B) were quantified using bands representing the mature form of [^{35}S]pb_2-DHFR protected against externally added proteinase K (+PK). Fig. 3C serves as a loading control for results shown in Fig. 3B. The total amount of [^{35}S]pb_2-DHFR associated with mitochondria was highest in the case of Δ*por1* mitochondria, lower with Δ*por2* mitochondria and lowest in the case of wild type mitochondria whereas

the amount of imported [^{35}S]pb$_2$-DHFR (PK-resistant fraction) was highest with wild type mitochondria, lower in the case of Δ*por2* mitochondria and lowest with Δ*por1* mitochondria. Further, the rate of the import, defined here as the contribution of the PK-resistant fraction to the total amount of [^{35}S]pb$_2$-DHFR associated with a given type of mitochondria (Fig. 3A), was highest in the case of wild type mitochondria, lower with Δ*por2* mitochondria and lowest in the case of Δ*por1* mitochondria. The differences in [^{35}S]pb$_2$-DHFR import rate disappeared after the removal of the outer membrane, i.e. in mitoplasts (not shown). Therefore, it might be concluded that upregulation of the TOM complex components results in higher levels of [^{35}S]pb$_2$-DHFR association with mitochondria, probably due to increased levels of its binding at the TOM complex, but the rate of subsequent translocation is markedly slowed down, probably because of involvement of the TOM complex in external NADH transport across the outer membrane under resting state conditions. The involvement seems to be deeper in Δ*por1* mitochondria than in Δ*por2* mitochondria, which correlates with the observed levels of TOM complex component upregulation.

Summing up, the obtained results point to a functional 'crosstalk' between the TOM complex channel and both isoforms of porin in mitochondria of *S. cerevisiae*. Thus, genetic or physiological elimination of porin isoform functions could cause significant interaction between metabolite transport and protein import into mitochondria due to the contribution of the TOM complex to metabolite transport.

Acknowledgements: The authors wish to thank Prof. M. Forte for the yeast strains as well as Prof. W. Neupert for *Escherichia coli* strain, pb$_2$-DHFR constructs and antisera. The technical assistance of D. Drachal-Chrul is gratefully acknowledged.

References

[1] Schein, S.J., Colombini, M. and Finkelstein, A. (1976) J. Membr. Biol. 30, 99–120.
[2] Colombini, M. (1979) Nature 279, 643–645.
[3] Benz, R. (1994) Biochim. Biophys. Acta 1197, 167–196.
[4] Colombini, M., Blachly-Dyson, E. and Forte, M. (1996) in: Ion Channels (Narahashi, T., Ed.), Vol. 4, pp. 169–201, Plenum Press, New York.
[5] Blachly-Dyson, E., Zambrowicz, E.B., Yu, W.H., Adams, V., McCabe, E.R.B., Adelman, J., Colombini, M. and Forte, M. (1993) J. Biol. Chem. 268, 1835–1841.
[6] Blachly-Dyson, E., Song, J., Wolfgang, W.J., Colombini, M. and Forte, M. (1997) Mol. Cell. Biol 17, 5727–5738.
[7] Elkeles, A., Breiman, A. and Zizi, M. (1997) J. Biol. Chem. 272, 6252–6260.
[8] Sampson, M.J., Lovell, R.S. and Craigen, W.J. (1997) J. Biol. Chem. 272, 18966–18973.
[9] Xu, X., Decker, W., Sampson, M.J., Craigen, W.J. and Colombini, M. (1999) J. Membr. Biol. 170, 89–102.
[10] Lee, A.C., Xu, X., Blachly-Dyson, E., Forte, M. and Colombini, M. (1998) J. Membr. Biol. 161, 173–181.
[11] Michejda, J., Kmita, H., Stobienia, O., Budzińska, M. and Lauquin, G.J.-M. (1994) in: Molecular Biology of Mitochondrial Transport System (Forte, M. and Colombini, M., Eds.), pp. 341–356, Springer-Verlag, Berlin.
[12] De Vries, S. and Marres, C.A.M. (1987) Biochim. Biophys. Acta 895, 205–239.
[13] Guo, X.J. and Lauquin, G.J.-M. (1986) EBEC Rep. 4, 292.
[14] Dihanich, M., Suda, K. and Schatz, G. (1987) EMBO J. 6, 723–728.
[15] Michejda, J., Guo, X.J. and Lauquin, G.J.-M. (1990) Biochem. Biophys. Res. Commun. 171, 354–361.
[16] Dihanich, M., Schmid, A., Oppliger, W. and Benz, R. (1989) Eur. J. Biochem. 181, 703–708.
[17] Stobienia, O., unpublished observation.
[18] Thieffry, M., Chich, J.F., Goldschmidt, D. and Henry, J.P. (1988) EMBO J. 7, 1449–1454.
[19] Fevre, F., Chich, J.F., Lauquin, G.J.-M., Henry, J.-P. and Thieffry, M. (1990) FEBS Lett. 262, 201–204.
[20] Hill, K., Model, K., Ryan, M.T., Dietmeier, K., Martin, F., Wagner, R. and Pfanner, N. (1998) Nature 395, 516–521.
[21] Künkele, K.-P., Heins, S., Dembowski, M., Nargang, F.E., Benz, R., Thieffry, M., Walz, J., Lill, R., Nussberger, S. and Neupert, W. (1998) Cell 93, 1009–1019.
[22] Künkele, K.-P., Juin, P., Pompa, C., Nargang, F.E., Henry, J.-P., Neupert, W., Lill, R. and Thieffry, M. (1998) J. Biol. Chem. 273, 31032–31039.
[23] Vallette, F.M., Juin, P., Pelleschi, M. and Henry, J.-P. (1994) J. Biol. Chem. 269, 13367–13374.
[24] Juin, P., Thieffry, M., Henry, J.-P. and Vallette, F.M. (1997) J. Biol. Chem. 272, 6044–6050.
[25] Kmita, H. and Budzińska, M. (2000) Biochim. Biophys. Acta 1509, 86–94.
[26] Antos, N., Stobienia, O., Budzińska, M. and Kmita, H. (2001) J. Bioenerg. Biomembr. (in press).
[27] Daum, G., Bohni, P.C. and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033.
[28] Douce, R., Bourguignon, R. and Neuberger, M. (1984) Methods Enzymol. 148, 403–415.
[29] Laemmli, U.K. (1970) Nature 227, 680–685.
[30] Wienhues, U., Koll, H., Becker, K., Guiard, B. and Hartl, F.-U. (1992) in: Protein Targeting. Practical Approach (Magge, A.I. and Wileman, T., Eds.), pp. 136–159, Oxford University Press, Oxford.
[31] Dekker, P.J., Martin, F., Maarse, A.C., Bömer, U., Müller, H., Guiard, B., Meijer, M., Rassow, J. and Pfanner, N. (1997) EMBO J. 16, 5408–5419.
[32] Voos, W., Gambil, B.D., Laloraya, S., Ang, D., Craig, D. and Pfanner, N. (1994) Mol. Cell. Biol. 14, 6627–6634.
[33] Rapaport, D., Mayer, A., Neupert, W. and Lill, R. (1998) J. Biol. Chem. 273, 8806–8813.
[34] Rapaport, D., Neupert, W. and Lill, R. (1997) J. Biol. Chem. 272, 18725–18731.
[35] Kamo, N., Muratsugu, M., Hongoh, R. and Kobatake, Y.J. (1979) J. Membr. Biol. 49, 105–121.
[36] Kerscher, O., Sepuri, N.B. and Jensen, R.E. (2000) Mol. Cell. Biol. 11, 103–116.
[37] Mayer, A., Neupert, W. and Lill, R. (1995) Cell 80, 127–137.
[38] Kmita, H., Stobienia, O. and Michejda, J. (1999) Acta Biochim. Pol. 46, 991–1000.
[39] Sirrenberg, Ch., Endres, M., Becker, K., Bauer, M.F., Walther, E., Neupert, W. and Brunner, M. (1997) J. Biol. Chem. 272, 29963–29966.