Most mitochondrial proteins are synthesized in the cytosol, imported into mitochondria via the TOM40 (translocase of the mitochondrial outer membrane 40) complex, and follow several distinct sorting pathways to reach their destination suborganellar compartments. Phosphate carrier (PiC) is an inner membrane protein with 6 transmembrane segments (TM1–TM6) and requires, after translocation across the outer membrane, the Tim9-Tim10 complex and the TIM22 complex to be inserted into the inner membrane. Here we analyzed an in vitro import of fusion proteins between various PiC segments and mouse dihydrofolate reductase. The fusion protein without TM1 and TM2 was translocated across the outer membrane but was not inserted into the inner membrane. The fusion proteins without TM1–TM4 were not inserted into the inner membrane but instead translocated across the inner membrane. Functional defects of Tim50 of the TIM23 complex caused either by depletion of the protein or the addition of anti-Tim50 antibodies blocked translocation of the fusion proteins without TM1–TM4 across the inner membrane, suggesting that lack of TM1–TM4 led to switch of its sorting pathway from the TIM22 pathway to the TIM23 pathway. PiC thus appears to have a latent signal for sorting to the TIM23 pathway, which is exposed by reduced interactions with the Tim9-Tim10 complex and maintenance of the import competence.

Newly synthesized organellar and secretory proteins are translocated across or inserted into biological membranes before they become functional. Among several different eukaryotic organelles, mitochondria are unique in being bounded by two biological membranes, the outer and inner mitochondrial membranes, rendering delivery of mitochondrial proteins to their destination suborganellar compartments highly complex. Mitochondria have translocators, membrane protein complexes that recognize targeting/sorting signals of mitochondrial proteins and facilitate their translocation across and/or insertion into the membranes. The outer membrane contains two TOM1 (translocase of the mitochondrial outer membrane) complexes as translocators, the TOM40 complex and TOB/SAM complex, and the inner membrane contains two TIM (translocase of the mitochondrial inner membrane) complexes as translocators, the TIM23 complex and TIM22 complex. The TOM40 complex mediates translocation across or insertion into the outer membrane for nearly all mitochondrial proteins. Most matrix proteins and some inner membrane proteins are synthesized in the cytosol as precursor proteins with an N-terminal cleavable presequence. After translocation through the TOM40 channel in the outer membrane, the proteins are passed onto the TIM23 complex and move through the TIM23 channel with the aid of MMC (mitochondrial Hsp70-associated motor and chaperone) proteins, i.e. mitochondrial Hsp70 and its partner proteins (4, 5). Polytopic inner membrane proteins, including metabolic carrier proteins, are synthesized in the cytosol without a presequence. After crossing the outer membrane via the TOM40 complex, these proteins are bound by the 70-kDa Tim9-Tim10 complex in the intermembrane space (IMS), which likely functions as a chaperone to prevent aggregate formation of the hydrophobic substrate proteins in the aqueous IMS (6, 7). Then the Tim9-Tim10 complex delivers the substrate proteins to the 300-kDa TIM22 complex consisting of the core complex (Tim22, Tim18, and Tim54) and the peripheral subunits (Tim9, Tim10, and Tim12), which facilitates insertion of substrate proteins into the inner membrane. The IMS contains many small and soluble proteins, including small Tim proteins that lack presequences. These small IMS proteins reach the IMS through the TOM40 channel with the aid of the inner membrane proteins Tim40/Mia40 (8, 9) and Hot13 (10). Although some single-membrane spanning outer membrane proteins are directly inserted into the outer membrane from the TOM40 complex, β-barrel outer membrane proteins first reach the IMS side of the outer membrane through the TOM40 complex and are then inserted into the outer membrane with the assistance of small Tim proteins in the IMS and the TOB/SAM complex in the outer membrane (11).

The TOM40 complex is thus the entry point for mitochondrial protein import pathways, which subsequently diverge from the TOM40 complex into distinct pathways for different sub-mitochondrial destinations. Then a question may arise as...
Protein Sorting between the TIM22 and TIM23 Pathways

MATERIALS AND METHODS

Plasmids—PiC-DHFR fusion proteins consist of PiC segments fused to mouse dihydrofolate reductase (DHFR). The PiC segments are named PiC[TM1-TM6], PiC[TM3-TM6], PiC[TM4-TM6], PiC[TM5-TM6]L, PiC[TM5-TM6]S, PiC[TM1-TM2], and PiC[TM3-TM4] for residues 208–311, residues 1–115, and residues 84–212 of PiC, respectively. PiC[TM1-TM6], PiC[TM3-TM6], PiC[TM4-TM6], PiC[TM5-TM6]L-DHFR, PiC[TM5-TM6]S-DHFR, PiC[TM1-TM2]-DHFR, PiC[TM3-TM4]-DHFR, and PiC[TM3-TM4]-DHFR.

FIG. 1. Structures of PiC and PiC-DHFR fusion proteins. Shown is a schematic diagram with TM segments (TM), (TM1-TM6)-DHFR, [TM3-TM6]-DHFR, [TM2-TM6]-DHFR, (TM4-TM6), [TM1-TM2]-DHFR, (TM3-TM4), and [TM3-TM4]-DHFR.

Import Assays—Radiolabeled precursor proteins were synthesized with rabbit reticulocyte lysate by coupled transcription/translation in the presence of [35S]methionine. Mitochondria were isolated from yeast strains D273-10B, W303-1A, and GAL-TIM10 (17) and incubated with radiolabeled precursor proteins in import buffer (600 mM sorbitol, 50 mM Hepes-KOH, pH 7.2, 80 mM KCl, 2.5 mM potassium Pi, 2 mM methionine, 5 mM MgCl2, 2 mM ATP, 2 mM NADH, and 1% bovine serum albumin) at 23 or 25 °C. Import reactions were stopped by addition of valinomycin to 10 μg/ml. The mitochondria were reisolated by centrifugation, and proteins were analyzed by SDS-PAGE and radioimaging.

RESULTS

Truncated PiC Segments Can Direct DHFR to the Matrix—To reveal the roles of internal segments of PiC in its import into mitochondria, we made DNA constructs for a series of PiC-DHFR fusion proteins between various PiC segments and DHFR (Fig. 1). We synthesized radiolabeled PiC-DHFR fusion proteins as well as PiC as a control with reticulocyte lysate and subjected them to in vitro import experiments with isolated yeast mitochondria. After incubation with mitochondria, PiC became resistant against PK added outside the mitoplasts (Fig. 2, PI, section labeled PiC lane, 2). Mitoplasts were generated from the mitochondria by rupturing the outer membrane and treating with PK, PiC was clipped and a protease-resistant fragment was generated, indicating its insertion into the inner membrane (Fig. 2, section labeled PiC lane, 4). This assembly process of PiC depends strictly on the mitochondrial potential across the inner membrane (Δψ), which is essential for the function of the TIM22 complex. Like PiC, PiC[TM1-TM6]-DHFR (the full-length PiC fused to DHFR) was sequestered to the protease-protected compartment after incubation with mitochondria in the presence of Δψ (Fig. 2A, section labeled [TM1-TM6] lane, 2). Because both the N terminus and the C terminus of PiC are exposed to the IMS (18), we expected that if PiC-DHFR fusion proteins were correctly sorted to the inner membrane like PiC, the DHFR part would be exposed to the IMS. The mitochondrial topology of the DHFR domain can be assessed by testing to determine whether protease added outside mitoplasts can cleave off the DHFR part and generate a protease-resistant PiC fragment in the inner membrane. Indeed, >70% of the imported fraction of PiC[TM1-TM6]-DHFR was degraded by PK added outside the mitoplasts to generate the protease-resistant 32-kDa PiC part (Fig. 2, A, section labeled [TM1-TM6], lane 4, white arrowhead, and B). PiC[TM1-TM6]-DHFR was thus imported into the IMS, and its PiC part was correctly inserted into the inner membrane, leaving the DHFR part exposed to the IMS.

2 T. Takahashi, unpublished results.
Protein Sorting between the TIM22 and TIM23 Pathways

**Fig. 2.** Import of PIC-DHFR fusion proteins into isolated mitochondria. A, radiolabeled PIC and PIC-DHFR fusion proteins were incubated with isolated mitochondria for 20 min at 25 °C in the presence (+) or absence (−) of Δψ. After the import, the samples were divided into halves, and one aliquot was subjected to osmotic swelling (SW) to generate mitoplasts. The mitochondria and mitoplasts were treated with 50 bars (5000 psi) for 30 min on ice. Black arrowheads indicate the positions expected for the PIC domains of each fusion protein, f, protease-resistant form of correctly assembled PIC in the inner membrane. B, the amounts of full-length fusion proteins, and asterisks indicate those proteins translated from an ATG codon other than the first one. White arrowheads indicate the positions expected for the PIC domains of each fusion protein. Lane 4 (white bars) and lane 4 (gray bars), which represent the fractions translocated across the outer and inner membranes, respectively. The amounts of radiolabeled proteins added to each reaction are set to 100%.

PIC[TM3-TM6]-DHFR (the PIC fragment lacking the first module fused to DHFR) and PIC[TM4-TM6]-DHFR (the PIC fragment starting from TM4 fused to DHFR) became protease-resistant in mitochondria, whereas 75 and 65%, respectively, of the imported fractions were degraded in mitoplasts and did not generate protease-resistant PIC fragments (Fig. 2, A and B, section labeled [TM4-TM6]). These results show that, in contrast to PIC[TM1-TM6]-DHFR, PIC[TM3-TM6]-DHFR and PIC[TM4-TM6]-DHFR were mainly (65–75%) imported into the IMS but were not correctly inserted into the inner membrane.

PIC[TM5-TM6]L-DHFR (the PIC fragment starting from the hydrophilic segment preceding TM5 fused to DHFR) and PIC[TM5-TM6]S-DHFR (the PIC fragment starting from TM5 fused to DHFR) were also imported into mitochondria (Fig. 2A, sections labeled [TM5-TM6]L and [TM5-TM6]S, lanes 2), but most (75–80%) of the imported fractions were protease-resistant even in mitoplasts (Fig. 2A, sections labeled [TM5-TM6]L and [TM5-TM6]S, lane 4). Protease-resistant PIC[TM5-TM6]L-DHFR and PIC[TM5-TM6]S-DHFR in both mitochondria and mitoplasts were degraded after solubilization with Triton X-100 (data not shown). These results suggest that PIC[TM5-TM6]L-DHFR and PIC[TM5-TM6]S-DHFR containing only one-third of PIC were imported into mitochondria and that ≈80% of the imported fraction unexpectedly directed the DHFR part to the matrix.

We next examined possible insertion of the imported PIC-DHFR fusion proteins into the inner membrane by alkaline extraction. Import of PIC and PIC[TM1-TM6]-DHFR were, like the integral membrane proteins Tim50, Tim17, Tim23, and endogenous PIC, recovered in the pellet fraction after Na2CO3 treatment and ultracentrifugation (Fig. 3), suggesting that they were correctly inserted into the inner membrane. On the other hand, imported PIC[TM3-TM6]-DHFR, PIC[TM4-TM6]-DHFR, PIC[TM5-TM6]L-DHFR, and PIC[TM5-TM6]S-DHFR were, like the β subunit of F1-ATPase (a peripheral membrane protein) and the soluble proteins cytochrome b5 and mitochondrial Hsp60, recovered in the supernatant fraction after Na2CO3 treatment and ultracentrifugation (Fig. 3). Therefore these fusion proteins lacking at least the first module were not correctly inserted into the inner membrane. Nevertheless, PIC[TM5-TM6]L-DHFR and PIC[TM5-TM6]S-DHFR were translocated completely across the inner membrane, whereas PIC[TM3-TM6]-DHFR and PIC[TM4-TM6]-DHFR mainly stayed outside the inner membrane, as described above.

The failure of PIC[TM3-TM6]-DHFR, PIC[TM4-TM6]-DHFR, PIC[TM5-TM6]L-DHFR, and PIC[TM5-TM6]S-DHFR in the assembly into the inner membrane may perhaps be due to the lack of the first and/or second modules, which might contain a correct sorting signal to the inner membrane. Because PIC[TM1-TM2]-DHFR, a fusion protein between the first module of PIC and DHFR, could not be imported into mitochondria efficiently (Fig. 2, A and B, section bars labeled [TM1-TM2]), we cannot test to determine whether the first module actually possesses a sorting signal for the TIM22 pathway. Nevertheless, PIC[TM3-TM4]-DHFR, a fusion protein between the second module of PIC and DHFR, was slightly imported into mitochondria but did not generate a protease-protected PIC part (Fig. 2, A and B, section bars labeled [TM3-TM4]). This result shows that at least the second module does not contain a correct sorting signal for the TIM22 pathway.

**Fusion Proteins with Truncated PIC Can Use the TIM23 Complex for Their Import—** By which pathway are the fusion proteins PIC[TM5-TM6]L-DHFR and PIC[TM5-TM6]S-DHFR delivered to the matrix, the TIM23 pathway or TIM22 pathway? To test the possible involvement of the TIM23 complex, we analyzed the in vitro import of PIC-DHFR fusion proteins...
into mitochondria isolated from Tim50-depleted (Tim50Δ) cells (16). Tim50 is a subunit of the TIM23 complex and is essential for protein translocation through the TIM23 channel. Mitochondria were isolated from GAL-TIM50 cells in which the TIM50 gene was under the control of the galactose-inducible GAL7 promoter after cultivation in a galactose-free medium at 23 °C for 13.5 h.

Because the translocation of presequence-containing precursor proteins through the TIM23 channel requires Tim50, the in vitro import of pSu9-DHFR (a fusion protein between the presequence of subunit 9 of F0-ATPase and DHFR) into Tim50Δ mitochondria was impaired as compared with that into wild-type (WT) mitochondria (Fig. 4A). In contrast, PiC, which uses the TIM22 complex instead of the TIM23 complex for its insertion into the inner membrane, was imported into Tim50Δ mitochondria as efficiently as into WT mitochondria (Fig. 4B). Although PiC(TM1-TM6)-DHFR was imported into both Tim50Δ mitochondria and WT mitochondria with similar efficiency (Fig. 4C), the import of PiC-DHFR fusion proteins with truncated PiC into mitochondria were affected more or less by the depletion of Tim50. The effects of the Tim50 deletion on in vitro import of the PiC-DHFR fusion proteins were in the order of PiC(TM4-TM6)-DHFR < PiC(TM3-TM6)-DHFR < PiC(TM5-TM6)-DHFR < PiC(TM5-TM6)-S-DHFR (Fig. 4, D–G). The import rate of PiC(TM5-TM6)-S-DHFR into Tim50Δ mitochondria was reduced to 45% of that into WT mitochondria.

The involvement of Tim50, i.e. the TIM23 pathway for the import of the [PiC]-DHFR fusion proteins, can also be assessed by testing the effects of anti-Tim50 antibodies on their import.
into mitoplasts. In mitoplasts where the outer membrane was selectively ruptured, antibodies can get access to Tim50 so that an antibody binding to Tim50 can block protein import via the FIG. 5.

Effects of anti-Tim50 antibodies on the import of PiC-DHFR fusion proteins into mitochondria and mitoplasts. A–G, mitochondria (filled circles) and mitoplasts (open circles) (25 μg) were preincubated with the indicated amounts of anti-Tim50 IgG in 200 μl of import buffer for 1 h at 4 °C. Radiolabeled pSu9-DHFR (A), Tim23 (B), and PiC-DHFR fusion proteins (C–G) were incubated with IgG-treated mitochondria or mitoplasts for 4 min (pSu9-DHFR) or 12 min (Tim23 and PiC-DHFR fusion proteins) min at 25 °C. The mitochondria and mitoplasts were treated with PK and recovered by centrifugation, and the proteins were analyzed by SDS-PAGE and radioimaging. The amount of protein imported into mitochondria without IgG was set to 100%. Treatment of mitochondria and mitoplasts with IgG prepared from the preimmune serum did not affect import of pSu9-DHFR (data not shown). Asterisks indicate those proteins translated from an ATG codon other than the first one.

FIG. 6. Effects of the depletion of Tim10 on the import of PiC-DHFR fusion proteins. A–G, mitochondria were isolated from yeast strains W303-1A (WT) and GAL-TIM10 (Tim10Δ) after cultivation in lactate (with 0.1% glucose) for 13 h at 23 °C. The amount of Tim10 in the Tim10Δ mitochondria was 7% of that in WT mitochondria. Radiolabeled pSu9-DHFR (A), PiC (B), and various PiC-DHFR fusion proteins (C–G) were incubated with WT mitochondria (filled circles) or Tim10Δ mitochondria (open circles) at 23 °C for the indicated times. The mitochondria were treated with PK, and the imported proteins were analyzed by SDS-PAGE and radioimaging. The amounts of radiolabeled proteins added to each reaction are set to 100%. Asterisks indicate those proteins translated from an ATG codon other than the first one.
Protein Sorting between the TIM22 and TIM23 Pathways

Fig. 7. Model of the sorting of PiC-DHFR fusion proteins between the TIM23 and TIM22 pathways. Translocation of PiC-DHFR fusion proteins through the TOM40 complex is facilitated by either the Tim9-Tim10 complex in the IMS or the TIM23 complex in the inner membrane. However, the fusion proteins with fewer TM segments are less dependent on the Tim9-Tim10 complex, and interactions with this complex are essential for sorting to the TIM22 pathway. The fusion proteins that are translocated across the outer membrane independently of the Tim9-Tim10 complex can probably be translocated through the TIM23 complex because of the latent TIM23 sorting signal in the PiC segments. 40, the TOM40 complex; 23, the TIM23 complex; 22, the TIM22 complex; 9–10, the Tim9-Tim10 complex; OM, the outer membrane; IMS, the inner membrane.

TIM23 complex (16). Although pSu9-DHFR was efficiently imported into mitoplasts, the import was significantly inhibited by the presence of anti-Tim50 antibodies (Fig. 5A) as shown previously (16). In contrast, the import of Tim23, a substrate for the TIM22 pathway, into mitoplasts was not blocked by anti-Tim50 antibodies (Fig. 5B). Because PiC strictly requires small Tim proteins for its insertion into the inner membrane, PiC (data not shown) and PiC[TM1-TM6]-DHFR (Fig. 5C) were not virtually imported into mitoplasts where substantial functions of soluble IMS proteins were lost (19). Although small fractions of PiC[TM3-TM6]-DHFR and PiC[TM4-TM6]-DHFR were imported into mitoplasts, the residual import into mitoplasts was impaired by the presence of anti-Tim50 antibodies. PiC[TM5-TM6]L-DHFR and PiC[TM5-TM6]S-DHFR were imported into mitoplasts with an efficiency of ∼50% of that into mitochondria, suggesting that these fusion proteins do not strictly require small and soluble Tim proteins in the IMS for their import into mitoplasts. However, the import of PiC[TM5-TM6]L-DHFR and PiC[TM5-TM6]S-DHFR into mitoplasts was significantly blocked by anti-Tim50 antibodies. Taken together, we conclude that, whereas PiC[TM1-TM6]-DHFR is inserted into the inner membrane via the TIM22 pathway, PiC[TM5-TM6]L-DHFR and PiC[TM5-TM6]S-DHFR move across the inner membrane via the TIM23 pathway. The import of PiC[TM3-TM6]-DHFR and PiC[TM4-TM6]-DHFR apparently represents situations between PiC[TM1-TM6]-DHFR and PiC[TM5-TM6]L-DHFR or PiC[TM5-TM6]S-DHFR. We also confirmed that anti-Tim23 antibodies impaired the import of pSu9-DHFR and PiC[TM5-TM6]S-DHFR into mitoplasts (data not shown).

Import of Fusion Proteins with Fewer PiC TM Segments Are Less Dependent on Tim10—Because different PiC-DHFR fusion proteins apparently require small Tim proteins differently, we analyzed the role of Tim10 in the import of PiC-DHFR fusion proteins. Mitochondria were isolated from GAL-TIM10 cells in which the TIM10 gene was under the control of the galactose-inducible GAL7 promoter after cultivation in galactose-free medium at 23 °C for 13 h. Although pSu9-DHFR was imported into both Tim10− mitochondria and WT mitochondria efficiently (Fig. 6A), the import of PiC and PiC[TM1-TM6]-DHFR into Tim10− mitochondria was significantly reduced to 15% of that into WT mitochondria (Figs. 6, B and C). This finding confirms that the import of PiC and PiC[TM1-TM6]-DHFR requires Tim10 in the IMS and is consistent with the fact that they are virtually not imported into mitoplasts without Tim10 (see above). On the other hand, the import rates of PiC[TM3-TM6]-DHFR, PiC[TM4-TM6]-DHFR, PiC[TM5-TM6]L-DHFR, and PiC[TM5-TM6]S-DHFR into Tim10− mitochondria were decreased to 50% of that into WT mitochondria (Fig. 6, D–G). Therefore, PiC[TM3-TM6]-DHFR, PiC[TM4-TM6]-DHFR, PiC[TM5-TM6]L-DHFR, and PiC[TM5-TM6]S-DHFR only partly require Tim10 for their import into mitochondria.

DISCUSSION

In the present study, we revealed that PiC, which is inserted into the inner membrane via the TIM22 pathway, has a latent ability to guide a passenger protein, DHFR, to the matrix via the TIM23 pathway. When fused to DHFR, the full-length PiC behaves like an authentic PiC and uses the Tim9-Tim10 complex in the IMS and the TIM22 complex to get itself inserted into the inner membrane (Fig. 7). Import or at least translocation across the outer membrane of the truncated PiC segments fused to DHFR (PiC[TM3-TM6]-DHFR, PiC[TM4-TM6]-DHFR, PiC[TM5-TM6]L-DHFR, and PiC[TM5-TM6]S-DHFR) requires the Tim9-Tim10 complex only partly (50% at most). After translocation across the outer membrane, PiC[TM5-TM6]L-DHFR and PiC[TM5-TM6]S-DHFR were efficiently translocated across the inner membrane and reached the matrix via the TIM23 pathway (Fig. 7). It is not clear if the TIM23 complex receives these fusion proteins only from the TOM40 complex or from the Tim9-Tim10 complex as well. PiC[TM9-TM10]-DHFR and PiC[TM4-TM6]-DHFR, which passed through the TOM40 channel, remained in the IMS but did not behave like integral membrane proteins. This means that, after translocation across the outer membrane, these fusion proteins cannot be inserted into the inner membrane with the aid of the TIM22 complex or cross the inner membrane through the TIM23 channel (Fig. 7).

What controls the sorting between the TIM23 pathway and the TIM22 pathway for the PiC-DHFR fusion proteins? A trivial possibility is that the DHFR has a cryptic sorting signal to the TIM23 pathway, which is exposed by the unfolding of the DHFR part upon translocation through the TOM40 channel and that this sorting signal competes with the sorting signal for the TIM22 pathway carried by the TM segments in the PiC part. However, this possibility seems unlikely, because PiC[TM5-TM6]L-DHFR and PiC[TM5-TM6]S-DHFR, whose DHFR domains are folded on the basis of the protease digestion
experiments (data not shown), could be imported into mito-
plasts (Fig. 5), whereas folded DHFR alone could not (data
not shown).

Presumably, the six hydrophobic TM segments of PiC tend to
aggregate and require chaperone functions of the Tim9-Tim10
complex to prevent aggregate formation (18, 20). Because PiC
and PiC[TM1-TM6]-DHFR have six TM segments, they are
efficiently associated with the Tim9-Tim10 complex and passed
on to the TIM22 complex. On the other hand, the fusion pro-
teins with a truncated PiC segment have fewer TM segments so
that the Tim9-Tim10 complex binds to them only partly. Ap-
parently, even the fractions of these proteins bound to the
Tim9-Tim10 complex are not productive transport intermedi-
ates and are not correctly transferred to the TIM22 complex.

What happens to the fractions of these proteins that passed the
outer membrane but are not associated with the Tim9-Tim10
complex? Although as much as 50% of PiC[TM5-TM6]L-DHFR
and PiC[TM5-TM6]S-DHFR were transferred to the TIM23
complex, only 20% of PiC[TM3-TM6]-DHFR and PiC[TM4-
TM6]-DHFR were received by the TIM23 complex (Fig. 5). This
result is likely due to the possibility that PiC[TM3-TM6]-
DHFR and PiC[TM4-TM6]-DHFR, which are not associated
with the Tim9-Tim10 complex, may form an aggregate or stick
nonspecifically to the membrane, thereby becoming transport-
incompetent in the IMS. Therefore, PiC apparently has a latent
sorting signal for the TIM23 pathway that is exposed if the PiC
segment is not concealed by the Tim9-Tim10 complex but still
remains import-competent. A precise determination of the PiC
segments recognized by the Tim9-Tim10 complex and the
TIM23 complex will help us to understand further the mecha-
nism of sorting between the TIM22 and TIM23 pathways.

Acknowledgments—We thank the members of the Endo Laboratory
for discussions and comments.

REFERENCES
1. Herrmann, J. M., and Neupert, W. (2000) Curr. Opin. Microbiol. 3, 210–214
2. Endo, T., Yamamoto, H., and Esaki, M. (2003) J. Cell Sci. 116, 3259–3267
3. Wiedemann, N., Frazier, A. E., and Pfanner, N. (2004) J. Biol. Chem. 279,
14473–14476
4. Neupert, W., and Brunner, M. (2002) Nat. Rev. Mol. Cell Biol. 3, 555–565
5. Rehling, P., Brandner, K., and Pfanner, N. (2004) Nat. Rev. Mol. Cell Biol. 5,
519–530
6. Koehler, C. M., Merchant, S., and Schatz, G. (1999) Trends Biochem. Sci. 24,
428–432
7. Bauer, M. P., Hofmann, S., Neupert, W., and Brunner, M. (2000) Trends Cell
Biol. 10, 25–31
8. Chacinska, A., Pfannschmidt, S., Wiedemann, N., Kozjak, V., Szklarz, L. K. S.,
Schulz-Specking, A., Truscott, K. N., Guiard, B., Meisinger, C., and Pfanner,
N. (2004) EMBO J. 23, 3735–3746
9. Naoe, M., Ohwa, Y., Ishikawa, D., Ohshima, C., Nishikawa, S., Yamamoto, H.,
and Endo, T. (2004) J. Biol. Chem. 279, 47815–47821
10. Curran, S. P., Leuenberger, D., Leverich, E. P., Hwang, D. K., Beverly, K. N.,
and Koehler, C. M. (2004) J. Biol. Chem. 279, 43744–43751
11. Pfanner, N., Wiedemann, N., Meisinger, C., and Lithgow, T. (2004) Nat.
Struct. Mol. Biology, 11, 1044–1048
12. Sirrenberg, C., Endres, M., Becker, K., Bauer, M. F., Wulber, E., Neupert, W.,
and Brunner, M. (1997) J. Biol. Chem. 272, 28663–28666
13. Rehling, P., Modek, K., Brandner, K., Kovermann, P., Sickmann, A., Meyer,
H. E., Kuhlbrandt, W., Wagner, R., Truscott, K. N., and Pfanner, N. (2003)
Science 299, 1747–1751
14. Esaki, M., Kanamori, T., Niishikawa, S., and Endo, T. (1999) Proc. Natl. Acad.
Sci. U. S. A. 96, 11770–11775
15. Kitada, K., Yamaguchi, E., and Aisawa, M. (1995) Gene 165, 203–206
16. Yamamoto, H., Esaki, M., Kanamori, T., Tamura, Y., Niishikawa, S., and Endo,
T. (2002) Cell 111, 519–528
17. Daum, G., Bohn, P. C., and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033
18. Wiedemann, N., Pfanner, N., and Ryan, M. T. (2001) EMBO J. 20, 951–960
19. Kührich, M., Rassow, J., Voss, W., Pfanner, N., Hölzeler, A. (1998) J. Biol.
Chem. 273, 16374–16381
20. Curran, S. P., Leuenberger, D., Oppliger, W., and Koehler, C. M. (2002) EMBO
J. 21, 942–953