The carboxyl-terminal Third of the Dicarboxylate Carrier Is Crucial for Productive Association with the Inner Membrane Twin-pore Translocase*

Received for publication, October 29, 2004, and in revised form, December 8, 2004
Published, JBC Papers in Press, December 9, 2004, DOI 10.1074/jbc.M412269200

Karin Brandner‡, Peter Rehling‡, and Kaye N. Truscott¶¶

From the ‡Institut für Biochemie und Molekularbiologie, Universität Freiburg, D-79104 Freiburg, Germany and the ¶Department of Biochemistry, La Trobe University, 3086 Melbourne, Victoria, Australia

The carrier proteins of the mitochondrial inner membrane consist of three structurally related tandem repeats (modules). Several different, and in some cases contradictory, views exist on the role individual modules play in carrier transport across the mitochondrial membranes and how they promote protein insertion into the inner membrane. Thus, by use of specific translocation intermediates, we performed a detailed analysis of carrier biogenesis and assessed the physical association of carrier modules with the inner membrane translocation machinery. Here we have reported that each module of the dicarboxylate carrier contains sufficient targeting information for its transport across the outer mitochondrial membrane. The carboxyl-terminal module possesses major targeting information to facilitate the direct binding of the carrier protein to the inner membrane twin-pore translocase and subsequent insertion into the inner membrane in a membrane potential-dependent manner. We concluded that, in this case, a single structural repeat can drive inner membrane insertion, whereas all three related units contribute targeting information for outer membrane translocation.

All nuclear-encoded mitochondrial proteins contain intrinsic targeting signals that direct them from their site of synthesis in the cytosol to their intended functional location. The nature of these signals, however, is not uniform from one protein to the next but falls into two main classes, either cleavable amino-terminal signal sequences (presequences) or multiple non-cleavable internal signals (1–7). The translocase of the outer mitochondrial membrane (TOM)1 complex recognizes and imports precursor proteins containing both types of targeting signal, whereas further import requires one of two translocases of the inner membrane (the TIM23 and TIM22 complexes) depending on the nature of the precursor protein (1, 3, 5–7). Precursors with a presequence are imported via the TIM23 complex, whereas precursors with internal targeting signals that are destined for the mitochondrial inner membrane are imported via the essential Tim9–10 complex of the intermembrane space and the TIM22 complex. The large hetero-oligomeric TIM22 complex is a twin-pore translocase (8) consisting of the integral membrane proteins Tim22, Tim54, and Tim18 and the peripheral membrane proteins of the small Tim family, Tim9, -10, and -12 (9–17).

A major representative of precursor proteins with internal targeting signals is the metabolite carrier protein family of the mitochondrial inner membrane. Carrier proteins fulfill vital functions for eukaryotic cells, as they are critical for metabolite exchange between mitochondria and the cytosol by forming a transport route across the tightly sealed inner mitochondrial membrane. Members of the carrier protein superfamily share common structural features; they are ~300 amino acids long and divided into three tandem repeats (modules) of similar length (18, 19). The structure of the mitochondrial ADP/ATP carrier (AAC) solved recently by x-ray crystallography revealed six transmembrane α-helices that form a barrel in the membrane (20). The structural fold of each repeat is similar and joined by two short loops exposed to the intermembrane space.

The question as to how the carriers themselves are transported to mitochondria and inserted into the inner membrane has drawn much attention. In vitro, the import of carrier proteins can be trapped at various stages along the way and has permitted a substantial dissection of the pathway (21–23). The newly synthesized carrier protein first binds to molecular chaperones in the cytosol (Stage I). The carrier protein then transfers to a major import receptor, Tom70, on the surface of mitochondria to which it remains bound in the absence of ATP (Stage II) (23–27). If the membrane potential (Δψ) across the inner membrane is fully dissipated but ATP is supplied, the carrier protein moves further along the import pathway crossing the outer membrane through the TOM complex and associates with the Tim9–10 complex of the intermembrane space (Stage III) (11–14, 23, 28). An association of the carrier protein with components of the inner membrane TIM22 complex is also detected under these conditions (8, 13, 29). If the Δψ is, however, only partially dissipated, the carrier precursor accumulates at the stage of inner membrane insertion associating with the TIM22 complex (Stage IV) (8). A full Δψ provides sufficient energy for the carrier precursor to insert into the inner membrane where subsequent assembly into a dimeric form occurs (Stage V) (23, 30).

Beyond this analysis, several studies, using different carrier proteins, fusions, and truncated constructs, have addressed the question of where the targeting information for the different transport steps resides within the precursor. It appears that the targeting determinants, which drive outer membrane receptor binding, translocation through the general import pore, and even association with members of the small Tim family, are contained within each structural repeat (29, 31, 32). At
least for receptor recruitment and outer membrane translocation, there is cooperation between each of these domains (31). Peptide binding scans of carriers revealed Tom70 and Tim9–10 binding sites within all three repeats (33–35). There is, however, controversy regarding the nature of carrier targeting signals that direct the later stages of carrier translocation from the intermembrane space to insertion into the inner membrane. Using the uncoupling protein 1, it was initially described that the first repeat alone can facilitate targeting and membrane insertion of a chimeric protein (36), whereas an independent study (37) using the same carrier reports that the middle repeat contains the complete targeting signal to direct association with the inner membrane translocation machinery and, hence, membrane insertion. Although the carboxyl-terminal two-thirds of AAC has been implicated in facilitating the later stages of import into mitochondria (38, 39), a more detailed study reports that the import signals that promote inner membrane insertion were specifically contained within the third module (29). In contrast to these data, a recent report suggests that the third module alone is insufficient to promote membrane insertion but rather that all three modules of the carrier precursor are required to allow membrane insertion via the TIM22 complex (32). Surprisingly, truncated forms of AAC fused to mouse dihydrofolate reductase were unable to follow the carrier transport pathway via the Tim9–10 and TIM22 complexes but were instead mistargeted to the presequence translocase (TIM23 complex), which directs them into the matrix (32). However, the major drawback of these studies, collectively, has been the lack of an experimentally accessible translocation intermediate during insertion into the inner membrane, i.e. a transport intermediate accumulated at the TIM22 complex. Thus, despite all efforts the role of the different segments of the carrier in protein insertion into the inner membrane has remained enigmatic.

Recently, by manipulation of the Δρ across the inner membrane, the precursor of the dicarboxylate carrier 1 (DIC) could be accumulated at the inner membrane TIM22 translocase (Stage IV) (8). This observation provided, for the first time, the possibility of directly analyzing the molecular determinants responsible for the late stages of carrier import leading to membrane insertion. We generated DIC constructs containing each module either singularly or in pairs. We showed that the third module of DIC contains the dominant targeting signals that allow binding to the twin-pore translocase and promotion of import from Stage III to V.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—Previously described yeast strains YPH499 (WT) (Matα ade2–101 his3–200 leu2–1 ura3–52 trp1–94, 200 bp from the start codon ATG and the nucleotide sequence encoding the first few amino acids of the desired DIC construct were used. Carboxyl-terminal tailing study reports that the import signals that promote inner membrane insertion but rather that all three modules of the carrier precursor are required to allow membrane insertion via the TIM22 complex (32). Surprisingly, truncated forms of AAC fused to mouse dihydrofolate reductase were unable to follow the carrier transport pathway via the Tim9–10 and TIM22 complexes but were instead mistargeted to the presequence translocase (TIM23 complex), which directs them into the matrix (32). However, the major drawback of these studies, collectively, has been the lack of an experimentally accessible translocation intermediate during insertion into the inner membrane, i.e. a transport intermediate accumulated at the TIM22 complex. Thus, despite all efforts the role of the different segments of the carrier in protein insertion into the inner membrane has remained enigmatic.

Recently, by manipulation of the Δρ across the inner membrane, the precursor of the dicarboxylate carrier 1 (DIC) could be accumulated at the inner membrane TIM22 translocase (Stage IV) (8). This observation provided, for the first time, the possibility of directly analyzing the molecular determinants responsible for the late stages of carrier import leading to membrane insertion. We generated DIC constructs containing each module either singularly or in pairs. We showed that the third module of DIC contains the dominant targeting signals that allow binding to the twin-pore translocase and promotion of import from Stage III to V.
mitochondrial inner membrane, we made mutants of DIC containing one or two structural repeats, referred to as modules (Fig. 1A). Resistance to proteinase K added to the outside of mitochondria following import was used as a measure of precursor translocation across the outer membrane. Full-length and DIC mutants were efficiently synthesized in rabbit reticulocyte lysate in the presence of [35S]methionine/cysteine (Fig. 1B, lane 1). Next, lysates were mixed with wild-type yeast mitochondria in the presence or absence of a Δψ across the inner membrane. After incubation at 25 °C, mitochondria were left untreated or treated with proteinase K, reisolated, and then analyzed by SDS-PAGE. Radioactive full-length and mutant DIC were found associated with mitochondria in the presence and absence of a Δψ (Fig. 1B, lanes 2 and 3). Moreover, for both full-length and mutant DIC significant amounts were also recovered with protease-treated mitochondria both in the presence or absence of a Δψ (Fig. 1B, lanes 4 and 5), suggesting that they were efficiently imported across the outer mitochondrial membrane. To ensure that reisolated radioactive signals represented translocated precursor and not just an intrinsic resistance of each mutant to proteinase K, controls were performed. Following import of each DIC construct independently, mitochondria were solubilized with Triton X-100 and proteinase K-treated. Proteinase K-resistant precursor was recovered for constructs DIC I, DIC II, and DIC III (Fig. 1B, lanes 8 and 9), but not to the same level as obtained in intact mitochondria. Thus, all DIC constructs were able to translocate across the mitochondrial outer membrane both in the presence and absence of a Δψ.

Depletion of the Δψ, by addition of the potassium ionophore valinomycin, abolishes translocation of the precursor into the inner membrane. The precursor is arrested in transport at Stage III. Under these conditions the amount of precursor that is protected against proteinase K reflects the portion that has passed the outer membrane but is not yet inserted into the inner membrane. When a Δψ is present, the precursor is also transported across the outer membrane to a protease-protected environment but is additionally able to insert into the inner membrane. To determine the efficiency of translocation across the outer membrane under both Δψ conditions, a quantitation was performed. Surprisingly, all DIC mutants were transported across the outer membrane more efficiently than full-length DIC, particularly under conditions of a full Δψ (Fig. 1C, compare lane 1 with lanes 2–7). In agreement with published data (30), the import of full-length DIC into mitochondria in the absence of a Δψ was only marginally reduced compared with fully energized mitochondria (Fig. 1C, compare lanes 1 and 8). However, the Δψ-dependent import of the DIC mutants was variable, with the most prominent effect observed for module III in which there was a reduction of ~50% when the Δψ was fully dissipated as compared with full Δψ (Fig. 1C, compare lanes 4 and 11).

We concluded that all modules of DIC contain sufficient targeting information for import across the outer membrane into the intermembrane space in both fully energized or Δψ-dissipated mitochondria. Whereas translocation across the outer membrane was only partially Δψ-dependent, module III contains a strong Δψ-responsive element. The ability of each individual DIC module or module combination to direct insertion of carrier proteins into the inner membrane via the TIM22 complex under conditions of variable Δψ could thus be examined in intact mitochondria.

DIC Modules Form Stage IV Intermediates—Recent work has raised doubt whether individual carrier modules follow the carrier pathway by engaging with the TIM22 translocase once they have passed through the outer membrane (32). To determine whether any single or combined modules of DIC contain sufficient targeting information to drive the late stages of carrier import (from III to V), their ability to form a Stage IV intermediate was investigated. We recently showed that dur-

Fig. 1. All modules of DIC can translocate across the mitochondrial outer membrane. A, schematic diagram of DIC constructs consisting of one or two of the three modules of DIC. B, import into mitochondria. Isolated wild-type mitochondria were incubated with [35S]labeled DIC constructs for 15 min at 25 °C in the presence or absence of valinomycin (1 μM). Following import, mitochondria were divided equally. One set of samples was left untreated (lanes 2 and 3); the second set was treated with proteinase K (lanes 4 and 5). As a control, radiolabeled precursors were imported into fully energized mitochondria, solubilized with 0.5% Triton X-100, and either left untreated (lanes 6 and 7) or proteinase K-treated (lanes 8 and 9). Reisolated mitochondria and radiolabeled precursors (10% of added lysate, lane 1) were subjected to SDS-PAGE and analyzed by digital autoradiography. Bands marked with an asterisk most likely represent truncated products of DIC arising from internal initiations of translation. C, quantitation of mitochondrial import. Import reactions were performed as described for panel B. Quantitation of the digital autoradiograms from proteinase K-treated mitochondria was performed with ImageQuant 1.2 (Amersham Biosciences). The intrinsic proteinase K resistance of constructs DIC I, DIC II, and DIC III was subtracted from import signals. Bars indicate the S.E. of the means.
A labeled carrier constructs were imported into wild-type mitochondria (11, 13, 21–23, 28, 30). Based on data drawn from import studies on both AAC and DIC (8, 9), carrier precursor into a dimeric form (Stage V). This general scheme is consistent on BN-PAGE equivalent to a Stage IV intermediate. 35S-labeled DIC modules form putative Stage IV intermediates.

As expected, full-length DIC formed the high molecular mass Stage IV intermediate, as well as the characteristic low molecular mass Stage III intermediate (which dissociates from the TOM complex during the BN-PAGE run) (43) and fully imported dimeric DIC (Stage V) (Fig. 2B, lane 1). The most striking observation was that all DIC mutants containing module III formed a Stage IV intermediate (Fig. 2B, lanes 4, 6, and 7), whereas modules I and II alone failed to form a visible Stage IV complex (Fig. 2B, lanes 2 and 3). Interestingly, DIC modules I and II combined formed a stable Stage IV intermediate (Fig. 2B, lane 5).

Targeting Determinants for Stage IV Formation Predominantly Reside in Module III of DIC—We have shown previously that the full-length DIC precursor binds to the TIM22 complex tightly enough to survive isolation of the complex from mitochondria and subsequent BN-PAGE analysis. Thus, to investigate whether or not the high molecular mass complexes observed on BN-PAGE (Fig. 2B) represented an association of carrier mutants with the TIM22 complex as opposed to non-specific associations, we isolated the complex following import of the DIC mutants into mitochondria under conditions of variable membrane potential. Radiolabeled precursors of DIC mutants were accumulated in mitochondria carrying Protein A-tagged Tim18 in the presence (full and intermediate levels) and absence of a Δψ. Following protease K treatment to remove unimported precursor, the TIM22 complex was purified by affinity chromatography on IgG-Sepharose from reisolated mitochondria solubilized in digitonin buffer. Following cleavage of Tim18 from the Protein A tag, the isolated complex was analyzed by BN-PAGE. Indeed, the high molecular mass complexes observed on BN-PAGE (Fig. 2B) represented Stage IV intermediates of the isolated TIM22 complex containing the radiolabeled DIC mutant precursors (Fig. 3A, lanes 13–28). These data clearly show that DIC mutants containing module III (DIC111, DIC1111, and DIC1111) also as well as DIC111 associate with the TIM22 complex. Interestingly, even with a low background noise characteristic of an isolated protein complex on BN-PAGE, there was no evidence of an interaction between DIC1 or DIC111 with the TIM22 complex (Fig. 3A, lanes 5–12).

A quantitation was performed to investigate the relative efficiency of Stage IV formation of each DIC mutant under different Δψ conditions. Bearing in mind that the mutant DIC precursors had variable competency for crossing the outer membrane (Fig. 1C), we developed an experimental strategy (Fig. 3B, upper panel) to carefully determine the relative efficiency of Stage IV formation compared with the amount of carrier precursor that translocated across the outer membrane (Import control). In addition, because some of the DIC mutants may have dissociated from the TIM22 complex during the BN-PAGE run (Fig. 3A, low molecular mass signals, lanes 13–28), all quantitations were taken from SDS-PAGE analysis of isolated Stage IV DIC intermediates (Fig. 3B, upper panel). Consistent with our previous findings, full-length DIC formed Stage IV intermediates in a Δψ-responsive manner (Fig. 3B, lanes 1–4), forming the previously defined “tethered” (no Δψ) and “Docked” states (intermediate Δψ) (8). Under conditions of maximum Δψ, most full-length DIC moved beyond Stage IV to assemble into a functional dimeric form in the membrane. Of

![Diagram](http://www.jbc.org/.../fig2.jpg)

**Fig. 2.** DIC modules form putative Stage IV intermediates. A, schematic diagram indicating stages of carrier import into mitochondria. At Stage I the newly synthesized precursor is bound to cytosolic chaperones. In the absence of ATP the carrier becomes trapped at Stage II on the outer membrane import receptor, Tom70. If ATP is supplied but the Δψ is fully dissipated, the carrier can be chased from Tom70 to associate with the Tim9–10 complex of the intermembrane space, generating the Stage III intermediate. Under these conditions an association of the carrier precursor with the TIM22 complex is evident, forming an early Stage IV intermediate. A late or docked Stage IV intermediate can be generated most efficiently when the Δψ is at intermediate levels. A full Δψ promotes efficient membrane insertion and assembly of the carrier precursor into a dimeric form (Stage V). This general scheme is based on data drawn from import studies on both AAC and DIC (8, 9, 11, 13, 21–23, 28, 30). B, some DIC constructs form a high molecular complex on BN-PAGE equivalent to a Stage IV intermediate. 35S-labeled carrier constructs were imported into wild-type mitochondria (50 μg of protein) in the presence of 30 μM CCCP, treated with proteinase K, and solubilized in 1% digitonin buffer prior to analysis by BN-PAGE. Asterisk denotes nonspecific complexes.
all the single module DIC mutants, DICIII bound to the TIM22 complex with a capacity close to the level of full-length (Fig. 3B, lanes 13–16), whereas the structural equivalents DICI and DICII did not bind at all (Fig. 3B, lanes 5–12), consistent with the results described above (Figs. 2B and 3A). The binding pattern in response to Δψ was, however, different for DICIII compared with full-length as maximum association with the TIM22 complex occurred in the absence of Δψ (Fig. 3B, lane 16) rather than at an intermediate level. Interestingly, when modules I and II were combined a propensity to bind the TIM22 complex was reestablished (Fig. 3B, lanes 17–20). Although DICIII could also bind the TIM22 complex, its ability to bind in the absence of Δψ was lower than for DICIII. In addition, the pattern of binding in relation to the Δψ indicated that further translocation from Stage IV to V was seemingly retarded by the presence of module II because under conditions of full Δψ a significant portion of the carrier mutant remained bound to the TIM22 complex (Fig. 3B, lane 25). The pattern of binding in response to Δψ of the artificial carrier construct DICI,III resembled that of full-length DIC but with much reduced efficiency at intermediate levels of Δψ (Fig. 3B, lanes 21–24).

These data collectively provide strong evidence that the major targeting elements responsible for driving import from Stage III to IV are contained within module III of DIC. This targeting element responds to the Δψ in a manner similar to full-length DIC. The inability of DIC modules I and II to interact with the TIM22 complex can, to some degree, be overcome when these modules are combined.
The Δψ-sensitive Module III of DIC Is Not Sufficient for Full Inner Membrane Carrier Insertion—Following binding to the TIM22 complex, a carrier protein inserts into the membrane in a Δψ-dependent manner where it assembles into its functional dimeric form. Because the amount of Stage IV intermediate that isolated with the TIM22 complex generally decreased with the dimeric form. After import, mitochondria were divided in half, reisolated, and reassociated in either SEM buffer (mitochondria) or MOES buffer (mitoplasts). Both mitochondria (lanes 1–4) and mitoplasts (lanes 5–8) were treated with proteinate K and analyzed by SDS-PAGE and digital autoradiography. As a control, radiolabeled precursors were imported into fully energized mitochondria, solubilized with 0.5% Triton X-100, and either left untreated (lane 9) or treated with proteinate K (lane 10). Control immunodecoration with specific antisera of proteinate K-treated mitochondria and mitoplasts indicates swelling was complete (lanes 11 and 12). Quantitation of Δψ-dependent inner membrane insertion of DIC constructs. Import reactions were as described in the legend to panel A and under “Experimental Procedures.” The yield of membrane-inserted precursor was assessed relative to outer membrane-translocated precursor. For quantitations, radioactive signals obtained for DIC constructs in the absence of Δψ were subtracted from the signals obtained from energized mitochondria to obtain a true assessment of Δψ-dependent membrane insertion. Quantitations were performed with ImageQuant 1.2 (Amer sham Biosciences). Bars indicate the S.E. of the means.

5–7). At first glance it seemed apparent that all DIC mutants were at least partially inserted in the inner membrane (Fig. 4A, lanes 5–8); this was unexpected, at least for DICI and DICIII, which did not even reach Stage IV. A possible explanation for the observed protease resistance would be that mitoplasting was incomplete. However, this was not the case, as intermembrane space-exposed proteins Tim50 and Tim10 were fully degraded upon proteinate K treatment of swollen mitochondria, whereas control matrix-located Mge1 remained protease-protected (Fig. 4A, lanes 11 and 12). It was evident that some DIC constructs have resistance to proteinate K in Triton X-100-treated mitochondria (DIC, DICIII, and DICIII, Fig. 4A, lane 10) as do all DIC constructs in Δψ-depleted mitoplasts (Fig. 4A, lane 8) that represent nonspecific background signals, because a membrane potential is required for insertion of carrier proteins.

To obtain a true assessment of Δψ-dependent membrane insertion for each of the DIC constructs, a quantitation was performed taking into account the nonspecific radioactive signals detected in the absence of Δψ. In addition, for each Δψ variable the level of protein insertion was assessed relative to outer membrane-translocated precursor. As expected for full-length DIC, a large fraction of the precursor inserted into the membrane under conditions of full and moderate Δψ (Fig. 4B, lanes 1 and 2). Surprisingly, although DICIII formed the Stage IV intermediate reasonably well compared with full-length DIC
Mitochondrial Carrier Protein Import

6221

(Fig. 3B), this did not translate to efficient membrane insertion (Fig. 4B, lanes 10 and 11). Inefficient membrane insertion was also observed for DIC1n (Fig. 4B, lanes 13 and 14). If, however, an additional module were combined with module III, membrane insertion was significantly improved (Fig. 4B, lanes 16–21). In the case of DIC1n, it reached a level greater than that obtained for full-length (Fig. 4B, compare lanes 1–3 with 19–21). For all mutant precursors that contained module III, membrane insertion increased with the strength of \( \Delta \psi \).

In summary, the translocation of DIC from Stage IV to V as a minimum requires \( \Delta \psi \)-sensitive import signals contained within module III of the protein. The addition of a second structural repeat to module III, however, improves membrane insertion ~2–3-fold under conditions of full membrane potential.

**DISCUSSION**

To address the controversy regarding the location of targeting signals that direct inner membrane insertion of carriers, we used the knowledge that full-length DIC binds tightly to the TIM22 complex as a means to investigate which region or regions of DIC contain the targeting information necessary for recognition and stable binding to this inner membrane translocase. Our results clearly showed that the major targeting information that mediates translocation via the carrier pathway is contained within module III of DIC, as all mutants containing this module (DICIII, DIC1n, and DIC1n/III) associated with the TIM22 complex. Module III bound with greatest efficiency in the absence of \( \Delta \psi \). This may reflect a strong ability of this module to bind a component of the TIM22 complex. Individual modules I or II did not associate with the TIM22 complex under any condition investigated, clearly indicating that alone they do not contain sufficient targeting information for inner membrane translocation even though all DIC modules were shown to transport across the outer membrane. On the other hand, the double module mutant DIC1n/III retained an ability to form a Stage IV complex, indicating that an additional targeting element may be contained within the joining loop of modules I and II or, alternatively, that signals that are separated in the linear sequence of DIC come together to form a TIM22 complex-specific targeting signal.

Although module III of DIC contained sufficient targeting information for binding to the TIM22 complex under the full range of \( \Delta \psi \) conditions examined, the single module alone did not allow a very efficient or perhaps stable membrane insertion. If, however, a second module were attached to module III, i.e. the natural module II or the artificially attached module I, then insertion was 2–3-fold better with a full membrane potential. It is possible that modules I or II of DIC alone may not bind strongly enough to the TIM22 complex to withstand the isolation procedure employed and therefore could actually contain targeting information that is not detected using our method. However, determination of membrane insertion of DICI and DICII revealed that these mutant constructs failed to incorporate into the membrane, supporting our belief that alone they do not contain sufficient targeting determinants to drive the late stages of import. In contrast DICI retained an ability to insert into the membrane, albeit inefficiently. Thus, although module III of DIC may not exclusively direct the late stages of import, the targeting information contained within this module promotes the most productive import.

In summary, we have used a new approach to examine carrier translocation from Stage IV to V. Our data suggest that the dominant targeting signals for DIC are contained within module III. These results support the findings of Endres et al. (29), who report that module III of AAC directs insertion into the inner membrane in a strictly \( \Delta \psi \)-dependent manner. Taken together, it appears that a single structural repeat or module predominately drives inner membrane translocation of carriers from Stage III to V via the TIM22 translocase. This is in contrast to outer membrane translocation to which each independent carrier module can contribute. It remains to be seen whether the major findings obtained in this study apply to all members of the carrier family.

**Acknowledgements**—We thank N. Pfanner for discussions and advice, N. Wiedemann for experimental advice, and D.A. Dougan for critically reading the manuscript.

**REFERENCES**

1. Bauer, M. F., Hofmann, S., Neupert, W., and Brunner, M. (2000) Trends Cell Biol. 10, 25–31
2. Gabriel, K., Buchanan, S. K., and Lithgow, T. (2001) Trends Biochem. Sci. 26, 36–40
3. Jensen, R. E., and Dunn, C. D. (2002) Biochim. Biophys. Acta 1592, 25–34
4. Endo, T., Yamamoto, H., and Esaki, M. (2003) J. Cell Biol. 162, 1529–1537
5. Rehling, P., Pfanner, N., and Meisinger, C. (2003) J. Mol. Biol. 326, 639–657
6. Truscott, R. N., Brandner, K., and Pfanner, N. (2003) Curr. Biol. 13, R328–R337
7. Koehler, C. M. (2004) Annu. Rev. Cell Dev. Biol. 20, 309–335
8. Rehling, P., Model, K., Brandner, K., Kovermann, P., Sickmann, A., Meyer, H. E., Kühlbrandt, W., Wagner, R., Truscott, K. N., and Pfanner, N. (2003) Science 299, 1747–1751
9. Sirrenberg, C., Bauer, M. F., Giardi, B., Neupert, W., and Brunner, M. (1996) Nature 384, 582–585
10. Kerscher, O., Hölzer, J., Srinivasan, M., Leung, R. S., and Jensen, R. E. (1997) Cell Biol. Cell Biol. 139, 1663–1676
11. Koehler, C. M., Jarosch, E., Tokatlidis, K., Schmid, K., Schweyen, R. J., and Schatz, G. (1998) Science 279, 369–373
12. Koehler, C. M., Merchant, S., Oppélger, W., Schmidt, K., Jarosch, E., Delfini, L., Jenne, T., Schatz, G., and Tokatlidis, K. (1998) EMBO J. 17, 6477–6486
13. Sirrenberg, C., Endres, M., Fölsch, H., Stuart, R. A., Neupert, W., and Brunner, M. (1998) Nature 391, 912–915
14. Adam, A., Endres, M., Sirrenberg, C., Lottspeich, F., Neupert, W., and Brunner, M. (1999) EMBO J. 18, 313–319
15. Koehler, C. M., Murphy, M. P., Bally, N. A., Leuenerberger, D., Oppélger, W., Delfini, L., Junne, T., Schatz, G., and Or, E. (2000) Mol. Cell. Biol. 20, 1165–1173
16. Kerscher, O., Sepnari, N. B., and Jensen, R. E. (2001) Mol. Biol. Cell. 12, 103–116
17. Kovermann, P., Truscott, R. N., Giardi, B., Rehling, P., Sepnari, N. B., Müller, H., Jensen, R. E., Wagner, R., and Pfanner, N. (2002) Mol. Biol. Cell 9, 363–373
18. Walker, J. R., and Runswick, M. J. (1993) J. Bioenerg. Biomembr. 25, 435–446
19. Palmieri, F. (1994) FEBS Lett. 346, 48–54
20. Pehay-Peyrolula, E., Dahout-Gonzalez, C., Kabin, R., Trézaguet, V., Lauquin, G. J., and Brandolin, G. (2003) Nature 426, 39–44
21. Pfanner, N., and Neupert, W. (1987) J. Biol. Chem. 262, 7528–7536
22. Pfanner, N., Tropschug, M., and Neupert, W. (1997) J. Biol. Chem. 272, 815–823
23. Ryan, M. T., Müller, H., and Pfanner, N. (1999) J. Biol. Chem. 274, 20619–20627
24. Hines, V., Brandt, A., Griffiths, G., Horstmann, H., Brutsch, H., and Schatz, G. (1999) EMBO J. 18, 3123–3132
25. Steger, H. F., Sollner, T., Kieber, M., Dietmeier, K. A., Pfaller, R., Trulzsch, K. S., Tropschug, M., Neupert, W., and Pfanner, N. (1999) J. Cell Biol. 115, 2353–2363
26. Young, J. C., Hoogenraad, N. J., and Hartl, F. U. (2003) EMBO J. 22, 41–50
27. Junge, C., Hoogenraad, N. J., and Hartl, F. U. (2003) Cell 112, 41–50
28. Junge, C., Hoogenraad, N. J., and Hartl, F. U. (2003) Cell 112, 41–50
29. Junge, C., Hoogenraad, N. J., and Hartl, F. U. (2003) Cell 112, 41–50
30. Junge, C., Hoogenraad, N. J., and Hartl, F. U. (2003) Cell 112, 41–50
31. Junge, C., Hoogenraad, N. J., and Hartl, F. U. (2003) Cell 112, 41–50
The Carboxyl-terminal Third of the Dicarboxylate Carrier Is Crucial for Productive Association with the Inner Membrane Twin-pore Translocase
Katrin Brandner, Peter Rehling and Kaye N. Truscott

J. Biol. Chem. 2005, 280:6215-6221.
doi: 10.1074/jbc.M412269200 originally published online December 9, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M412269200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 43 references, 22 of which can be accessed free at
http://www.jbc.org/content/280/7/6215.full.html#ref-list-1