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 - For a mean composition of 12.7% carbon for ALH84001 carbonates, production of ^{14}C by spallation of oxygen, by irradiation of this material in a small object in space, must result in about 74 disintegrations per minute per kilogram (3.2×10^8 ^{14}C atoms per gram) (9, 10). This calculated ^{14}C activity corresponds to a $^{14}\text{C}/^{12}\text{C}$ ratio (atom/atom) of 5.0×10^{-14} or 4.3% of the ratio found in modern, pre-bomb carbon (~1950 A.D.). Using the composition of calcite for EETA79001 results in a value of 4% of that in modern carbon (9, 10, 17). After 13 and 12 ka (the terrestrial ages of these two meteorites), we would expect the ^{14}C in carbonates from these two meteorites to have decayed to a level about 0.9% of the modern value.
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 - A negligible amount of ^{14}C could have been produced by spallation reactions in the organic components of these meteorites. The amount produced by spallation would not produce a significant $^{14}\text{C}/^{12}\text{C}$ ratio. No appreciable ^{14}C should have been produced while in space in the organic phases through the action of cosmic-ray-generated secondary thermal neutrons on ^{14}N or through thermal neutron capture on ^{13}C . The reason is that secondary cosmic-ray neutrons can only become thermalized inside a large parent meteoroid or in a smaller body with significant water content. Given that all of the martian meteorites so far recovered were irradiated as small objects in space with little water content, very few cosmic-ray-generated neutrons can have been thermalized. M. S. Spiegel *et al.* [*Proc. Lunar Planet. Sci. Conf.* **16**, *J. Geophys. Res.* **91**, 483 (1986)] have shown that for objects of pre-atmospheric radius less than ~ 50 g/cm 2 (or approximately 19 kg in mass) that the cosmic-ray-induced thermal neutron flux is extremely small and neutron products are not detectable. ALH84001 and EETA79001 were much smaller than this size (recovered masses of 2.1 and 7.9 kg, respectively), and thus the thermal neutron flux would be even lower and we can rule out any significant thermal neutron production of ^{14}C in the organic components of these martian meteorites. Consequently, organic material indigenous to ALH84001 or EETA79001 would be expected to have very low ^{14}C abundance resulting from irradiation in space.
 - Carbonaceous material produced in equilibrium with the atmosphere after 1950 A.D. would contain higher levels of ^{14}C (up to twice the modern values) because of contamination of the atmosphere by nuclear testing [I. Levin *et al.*, *Radiocarbon* **27**, 1 (1985)].
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 - The carbon isotopic compositions are determined as
- $$\delta^{13}\text{C} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 10^3$$
- where the standard is Pee Dee belemnite.
- For each combustion, between 0.25 and 0.40 g of meteorite powder were placed in a cleaned 9-mm Vycor glass tube, which was then evacuated. Ultrapure oxygen was introduced at a pressure of ~ 0.3 atm. The samples were then combusted at a series of temperature steps each lasting 20 to 30 min. The temperature was controlled by a resistance furnace and thermocouple apparatus. After each step, the evolved CO_2 was cryogenically collected and cleaned by standard radiocarbon procedures (14, 32). An aliquot of oxygen was added to the cell between each step. The $\delta^{13}\text{C}$ of each gas was measured with a stable-isotope mass spectrometer, cryogenically recovered from the dual inlet, and then catalytically converted to graphite over iron (33) for ^{14}C analysis by AMS. The AMS measurements on these graphite targets were made at the University of Arizona AMS Facility (34). Blanks run at each temperature step showed 3 ± 1 μg of modern C for each extraction step (35). For our samples, each combustion step yielded between 9 and 79 μg of C, with an average sample size of ~ 30 μg .
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 - The blank levels we determined for a series of combustion steps gave 3 μg (75° to 200°C), 2.5 μg (200° to 400°C), and 5.6 μg (400° to 600°C) of modern carbon. Other studies on blanks in our laboratory for combustions using this line give a mean blank of 3 ± 1 μg of carbon.
 - We are grateful to the Meteorite Working Group for provision of the samples. We wish to thank A. L. Hatheway, D. Biddulph, L. R. Hewitt, and T. E. Lange for technical assistance, and K. Hutchins, G. S. Burr, D. J. Donahue, and C. J. Eastoe for many useful scientific discussions. This work was partly supported by NASA grant NAGW-3614 and NSF grant EAR 95-08413.

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Import of Mitochondrial Carriers Mediated by Essential Proteins of the Intermembrane Space

Carla M. Koehler, Ernst Jarosch, Kostas Tokatlidis, Karl Schmid, Rudolf J. Schweyen, Gottfried Schatz*

In order to reach the inner membrane of the mitochondrion, multispanning carrier proteins must cross the aqueous intermembrane space. Two essential proteins of that space, Tim10p and Tim12p, were shown to mediate import of multispanning carriers into the inner membrane. Both proteins formed a complex with the inner membrane protein Tim22p. Tim10p readily dissociated from the complex and was required to transport carrier precursors across the outer membrane; Tim12p was firmly bound to Tim22p and mediated the insertion of carriers into the inner membrane. Neither protein was required for protein import into the other mitochondrial compartments. Both proteins may function as intermembrane space chaperones for the highly insoluble carrier proteins.

Most proteins imported to mitochondria are synthesized with a cleavable NH_2 -terminal targeting sequence and are sorted to their correct intramitochondrial location by the dynamic interaction of distinct transport systems in the outer and inner membranes (1). The TIM system in the inner membrane consists of two integral membrane proteins, Tim17 and Tim23, which make up the inner membrane import channel. Complete translocation into the matrix is coupled to adenosine triphosphate (ATP)

hydrolysis and is mediated by Tim44, mHsp70, and GrpE. However, some of the most abundant inner membrane proteins, such as the metabolite carriers, are synthesized without a cleavable NH_2 -terminal presequence and therefore do not engage with the Tim23 channel. It has been suggested that import of these proteins is directed by one or more internal targeting signals (2), but the exact mechanism is still poorly defined. In the cytosol of the yeast *Saccharomyces cerevisiae*, chaperones escort these insoluble carrier proteins preferentially to the outer membrane receptors Tom37 and Tom70 (3). The carriers then move through the TOM channel in the outer membrane and insert into the inner membrane, bypassing the ATP-dependent Tim23 system, which transports proteins

C. M. Koehler, K. Tokatlidis, K. Schmid, G. Schatz, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.
E. Jarosch and R. J. Schweyen, Institut für Mikrobiologie und Genetik, University of Vienna, A-1030 Vienna, Austria.

*To whom correspondence should be addressed. E-mail: schatz@ubaclu.unibas.ch

across the inner membrane (4, 5).

Here we report that the transfer of insoluble carrier proteins from the TOM system of the outer membrane across the aqueous intermembrane space to the inner membrane is mediated by Tim10p and Tim12p, two cysteine-rich proteins of the intermembrane space. The two proteins are 35% identical and are essential for viability. They were discovered as multicopy suppress-

sors of mitochondrial RNA splicing defects and were initially termed Mrs11p and Mrs5p (6–8).

To identify the function of Tim10p and Tim12p, we constructed temperature-sensitive mutants by *in vitro* mutagenesis (9). Haploid yeast cells expressing a temperature-sensitive *tim10-1* or *tim12-1* protein grew at wild-type rates at 25°C but stopped growing 6 to 8 hours after a shift to 37°C on

fermentable as well as nonfermentable carbon sources. Return to 25°C did not restore growth (10). Mitochondria purified from heat-arrested *tim10-1* cells contained drastically reduced steady-state concentrations of several carriers located in the inner membrane—including the ADP-ATP carrier (AAC), the phosphate carrier (PiC), and the dicarboxylate carrier (DiC) (11)—as well as of Tim22p (12) (Fig. 1A). A similar but milder defect was found in mitochondria from the heat-arrested *tim12-1* mutant, with the exception of Tim22p, which was not detectable. Both arrested mutants had wild-type levels of proteins sorted to the outer membrane, matrix, intermembrane space, and inner membrane that were unrelated to the carrier family. Thus, the defect in both mutants was specific for the metabolite carrier family and for Tim22p.

To determine whether the import of carriers was impaired, we tested mitochondria from the heat-arrested mutants for their ability to import radiolabeled precursors synthesized *in vitro* (13) (Fig. 1B). Import was assessed by protection from added protease, which removes nonimported precursor. The observed import defects mirrored the deficiency in steady-state levels: import of AAC and PiC was inhibited by 85% in *tim10-1* mitochondria and by 50% in *tim12-1* mitochondria, whereas import of Tim22p was inhibited by 85% in both *tim10-1* and *tim12-1* mitochondria. All of the proteins whose import into isolated mitochondria was affected by the two mutations lack a cleavable targeting signal. In contrast, import or proteolytic processing of mitochondrial precursor proteins with a cleavable NH₂-terminal targeting pre-

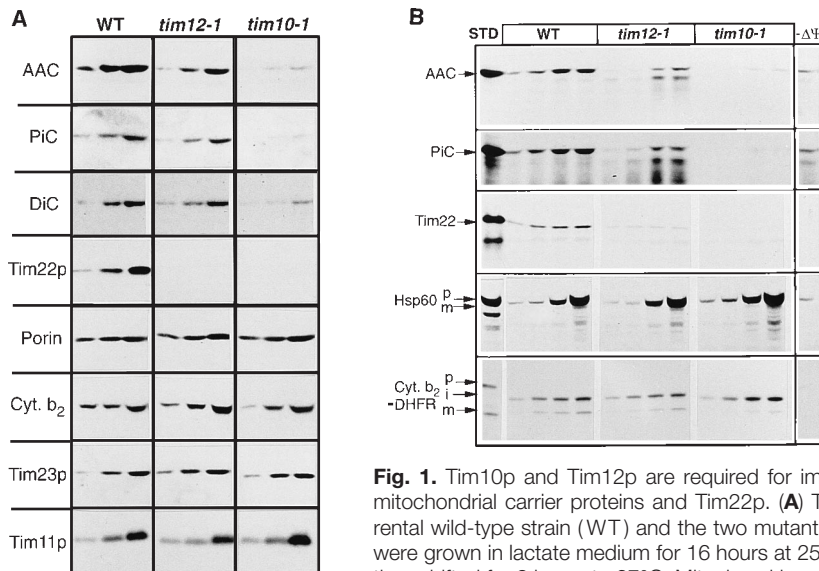
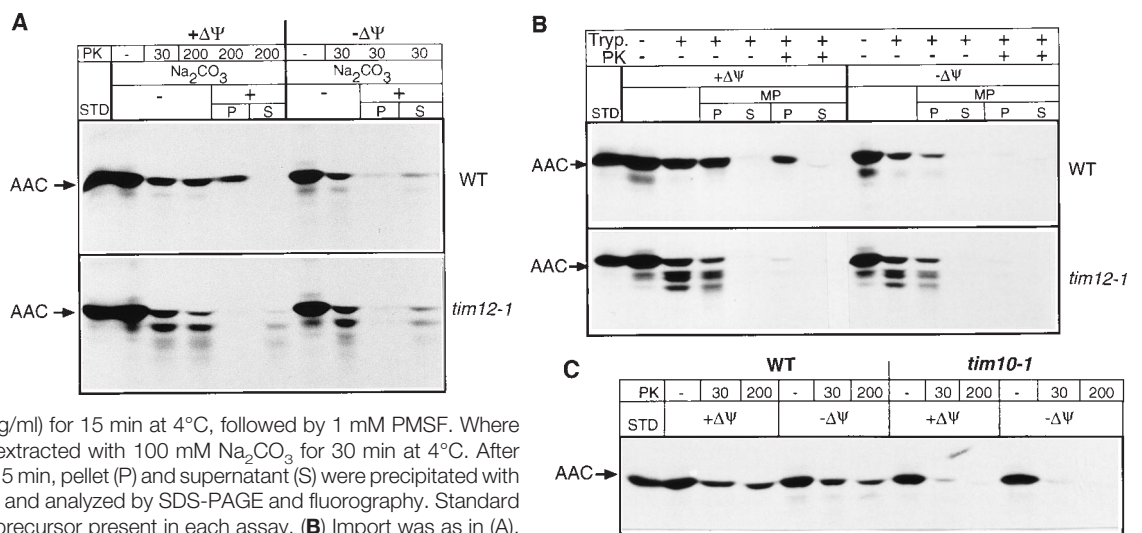


Fig. 1. Tim10p and Tim12p are required for import of mitochondrial carrier proteins and Tim22p. (A) The parental wild-type strain (WT) and the two mutant strains were grown in lactate medium for 16 hours at 25°C and then shifted for 8 hours to 37°C. Mitochondria were isolated and analyzed by SDS-PAGE and immunoblotting

with monospecific rabbit antisera for AAC, porin, cytochrome b₂, and Tim11p (20, 40, and 80 μg loaded) and PiC, DiC, and Tim22p or Tim23p (50, 100, and 200 μg loaded). Blots were decorated with [¹²⁵I]-protein A. (B) Precursors were synthesized *in vitro* and incubated at 25°C with fully energized wild-type or mutant mitochondria, followed by protease K treatment, SDS-PAGE, and fluorography (13). Aliquots were analyzed for import after 2.5, 5, 10, and 15 min; p, precursor; i, intermediate form; m, mature form; -ΔΨ, absence of a membrane potential; import for 15 min into wild-type mitochondria uncoupled by 25 μM FCCP.

Fig. 2. Tim12p is required for insertion of AAC into the inner membrane, whereas Tim10p is required for translocation of the AAC across the outer membrane.

(A) AAC was synthesized *in vitro* and incubated with wild-type (WT) and *tim12-1* mitochondria for 8 min at 25°C in the presence or absence of a membrane potential (ΔΨ). Mitochondria were then divided into equal aliquots that were either left untreated (-) or treated with proteinase K (PK, 30 or 200 μg/ml) for 15 min at 4°C, followed by 1 mM PMSF. Where indicated, mitochondria were extracted with 100 mM Na₂CO₃ for 30 min at 4°C. After centrifugation at 100,000g for 15 min, pellet (P) and supernatant (S) were precipitated with 10% trichloroacetic acid (TCA) and analyzed by SDS-PAGE and fluorography. Standard (STD): 10% of the radioactive precursor present in each assay. (B) Import was as in (A). The designated samples were then treated with trypsin (100 μg/ml) for 30 min at 4°C, followed by addition of trypsin inhibitor (200 μg/ml). Two aliquots were converted to mitoplasts (MP) in the absence or presence of proteinase K (50 μg/ml), separated into pellet (P) and supernatant (S), and then analyzed as in (A). (C) Import into *tim10-1* mitochondria was as in (A). After import, three equal aliquots were taken: one was left untreated (-), whereas the other two were treated with proteinase K (30 or 200 μg/ml) for 15 min at 4°C, followed by addition of 1 mM PMSF. All samples were then analyzed as in (A).



quence was unaffected by either mutation, regardless of whether the precursors were targeted to the intermembrane space or to the matrix.

Import of the AAC precursor into wild-type mitochondria occurs in several discrete stages (5). In stage 1, the soluble carrier, escorted by cytosolic chaperones, binds to the mitochondrial import receptors. In stage 2, it partially (and probably reversibly) inserts across the TOM pore in the outer membrane, becoming partly inaccessible to proteinase K. In stage 3, the carrier accumulates on the outer surface of the inner membrane, becoming inaccessible to proteinase K in intact mitochondria, but not in mitoplasts (mitochondria whose outer membrane has been selectively ruptured). In stage 4 (which requires an electric potential across the inner membrane), the carrier inserts fully into the inner membrane and is then unable to be extracted with alkali, with only a small NH₂- or COOH-terminal part accessible to protease in mitoplasts. In stage 5, dimerization of the inserted carrier in the inner membrane completes the process.

To determine the specific stage at which the import of carriers was blocked in the *tim10-1* and *tim12-1* mutants, we allowed mitochondria from the heat-arrested cells to import AAC and then either alkali-extracted or converted them to mitoplasts in the presence of protease. In agreement with published results, AAC inserted into the inner membrane of wild-type mitochondria only in the presence of an inner membrane potential, the inserted protein was resistant to protease in mitochondria and mitoplasts, and the fully inserted carrier was not extracted by alkali (Fig. 2, A and B). In contrast, import of the carrier into mitochondria from heat-arrested *tim12-1* cells was blocked at stage 3 even in the presence of an inner membrane potential: the imported carrier was resistant to protease in intact mitochondria (Fig. 2A) but not in mitoplasts (Fig. 2B) and remained largely alkali-extractable (Fig. 2A). (Nonspecific losses of alkali-extractable AAC most likely were caused by nonspecific adherence to the tube wall.) Import of multispanning carrier proteins into fully energized *tim12-1* mitochondria thus resembled the incomplete import into wild-type mitochondria lacking an inner membrane potential.

Import of AAC into mitochondria from the heat-arrested *tim10-1* mutant was blocked at an earlier stage than import into *tim12-1* mitochondria: the precursor was bound to the mitochondria, but most of it remained protease-accessible (Fig. 2C), and what little was imported remained alkali-extractable (11). The import block caused by loss of Tim10p function thus occurs be-

fore or at stage 2, suggesting that Tim10p is required for transfer of the carrier across the outer membrane.

To show directly that Tim10p interacted with AAC during import, we imported radiolabeled AAC into fully energized or uncoupled mitochondria, removed nonimported protein by protease treatment, reacted the mitochondria with a cleavable cross-linker, and analyzed the radioactive cross-linked products by SDS-polyacrylamide gel electrophoresis (PAGE) and fluorography (14). Import into fully energized wild-type mitochondria yielded only a low percentage of cross-linked products, presumably because the imported carrier was rapidly inserted into the inner membrane (Fig. 3A). Little cross-linking was detected with mitochondria from the *tim10-1* mutant (Fig. 3A), because the carrier was not transported across the outer membrane. In contrast, at least two cross-linked products were found

after import into fully energized or uncoupled *tim12-1* mitochondria or into uncoupled wild-type mitochondria. Each of the two cross-linked products was as abundant as the non-cross-linked AAC, and their apparent masses were 10 and 20 kD greater than that of AAC. Both products could be immunoprecipitated by monospecific antiserum against Tim10p in uncoupled wild-type mitochondria (Fig. 3B) and *tim12-1* mitochondria (11), indicating that they contained Tim10p. Upon cleavage with 2-mercaptoethanol, the cross-linked species yielded only the radioactive 34-kD band of AAC (11). Thus, loss of Tim12p function, or uncoupling of wild-type mitochondria, caused accumulation of Tim10p-bound AAC inside the outer membrane. The large cross-linked bands may contain Tim12p, but antibodies against Tim12p could not establish this point with certainty.

To identify proteins that interact with

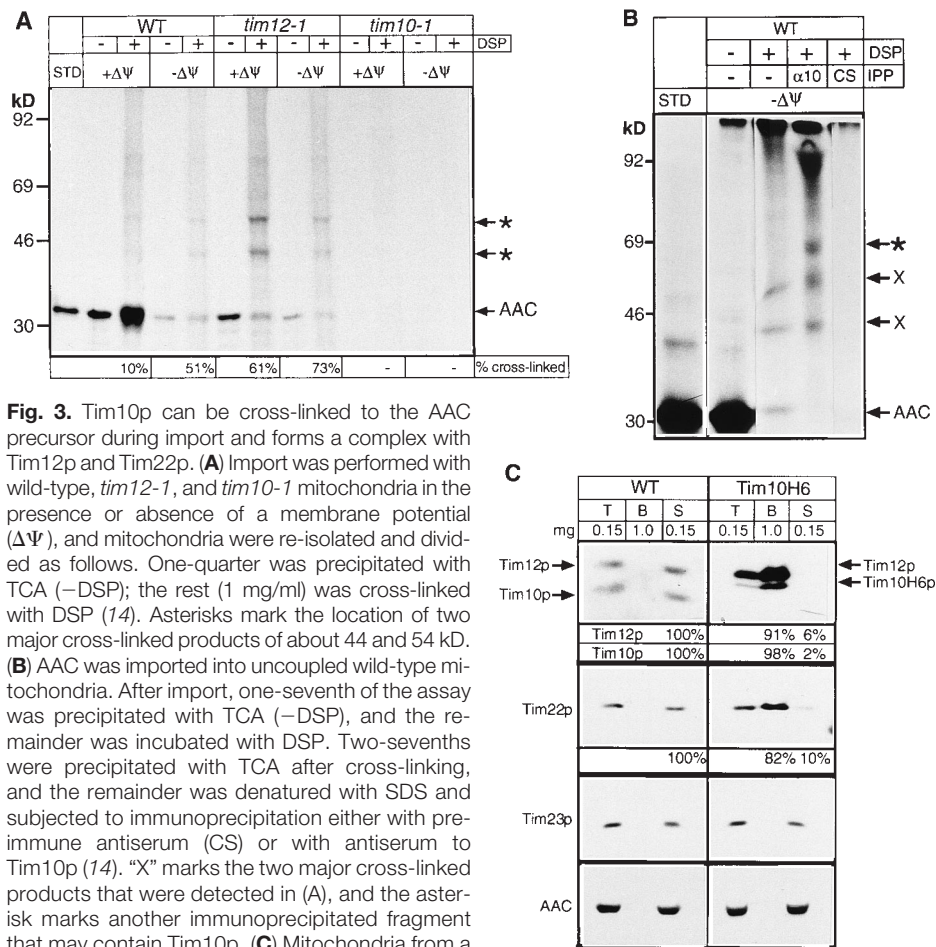


Fig. 3. Tim10p can be cross-linked to the AAC precursor during import and forms a complex with Tim12p and Tim22p. (A) Import was performed with wild-type, *tim12-1*, and *tim10-1* mitochondria in the presence or absence of a membrane potential ($\Delta\Psi$), and mitochondria were re-isolated and divided as follows. One-quarter was precipitated with TCA (-DSP); the rest (1 mg/ml) was cross-linked with DSP (14). Asterisks mark the location of two major cross-linked products of about 44 and 54 kD. (B) AAC was imported into uncoupled wild-type mitochondria. After import, one-seventh of the assay was precipitated with TCA (-DSP), and the remainder was incubated with DSP. Two-sevenths were precipitated with TCA after cross-linking, and the remainder was denatured with SDS and subjected to immunoprecipitation either with pre-immune antiserum (CS) or with antiserum to Tim10p (14). "X" marks the two major cross-linked products that were detected in (A), and the asterisk marks another immunoprecipitated fragment that may contain Tim10p. (C) Mitochondria from a strain expressing a COOH-terminal hexahistidine-tagged Tim10p [Tim10H6p (14)] were solubilized at 2 mg/ml by 0.5% digitonin. As a control, 150 μ g of extract was withdrawn (T), and 1 mg was incubated with Ni²⁺-agarose beads. The beads were washed, and bound proteins were eluted with 1% SDS (B). To assess the effectiveness of binding, 150 μ g of the unbound proteins (S) was also analyzed. Proteins co-isolating with the hexahistidine-tagged Tim10p were identified and quantified by immunoblotting. The fraction of bound and unbound protein is given below each panel. Mitochondria from the parental wild-type strain were analyzed as a negative control.

Tim10p and Tim12p, we searched for yeast genes whose overexpression abrogated the conditional lethality of the *tim10-1* and *tim12-1* mutations (15). No suppressor was identified for the *tim10-1* mutation, but we isolated one strong suppressor for the *tim12-1* mutation. After subcloning, this suppressor proved to be the *TIM22* gene. Overexpression of *TIM22* allowed the *tim12-1* mutant to grow at the restrictive temperature 37°C but was without effect on the conditional lethality of the *tim10-1* mutant or a *tim12* null strain (11). None of the genes for other known components of the mitochondrial import system, including *TIM23* or *TIM17*, were identified in this multicopy suppressor screen. Even though Tim10p and Tim12p are 35% identical, overexpression of one protein did not suppress the lethality caused by the temperature sensitivity of the other (11).

The results presented so far suggested that Tim10p, Tim12p, and Tim22p interact functionally and genetically. To test for a physical interaction between Tim10p, Tim12p, and Tim22p, we constructed a yeast strain in which all Tim10p molecules had a COOH-terminal hexahistidine tag (16). This strain grew as well as the parental wild type under all conditions tested, suggesting that the tag did not abolish the function of Tim10p in vivo. Essentially all of the tagged Tim10p present in digitonin-solubilized mitochondria was recovered on the Ni²⁺-agarose beads (Fig. 3C). More than 90% of the Tim12p and 80% of the Tim22p present in the mitochondrial lysate co-purified with the tagged Tim10p. No such co-purification was detected if Tim10p was untagged, and inner membrane proteins such as AAC, Tim23p, Tim11p, Tim44p, and Afg3p (11, 17) did not co-

purify with the tagged Tim10p (Fig. 3C). Thus, the genetic and biochemical data indicated that Tim10p, Tim12p, and Tim22p are three components of a multisubunit complex that mediates import and insertion of multispinning carrier proteins into the inner membrane.

Tim10p and Tim12p perform distinct functions in the import of mitochondrial carriers because overexpression of Tim10p did not substitute for loss of Tim12p and vice versa. Tim10p acted before Tim12p: Loss of Tim10p function blocked transport across the outer membrane, whereas loss of Tim12p function blocked insertion into the inner membrane. Tim10p was only loosely associated with the complex because most of it was solubilized when mitochondria were converted to mitoplasts (7). Association of Tim10p with the inner membrane may be reversible, allowing Tim10p to (i) bind precursor whose COOH-terminus is still in the outer membrane channel or exposed on the outer surface and (ii) transfer the bound precursor to Tim12p. We suggest the following model for the import pathway of a multispinning carrier from the mitochondrial surface into the inner membrane (Fig. 4). The carrier, after binding to mitochondrial import receptors, passes through the outer membrane pore and binds to Tim10p; this step may be reversible. The carrier is then transferred to Tim12p, which is associated with Tim22p on the outer face of the inner membrane. The membrane-embedded Tim22p finally mediates the correct insertion of the carrier into the inner membrane.

Because Tim10p and Tim12p are required for insertion of Tim22p, which in turn is required for the insertion of carrier proteins, Tim10p and Tim12p might in

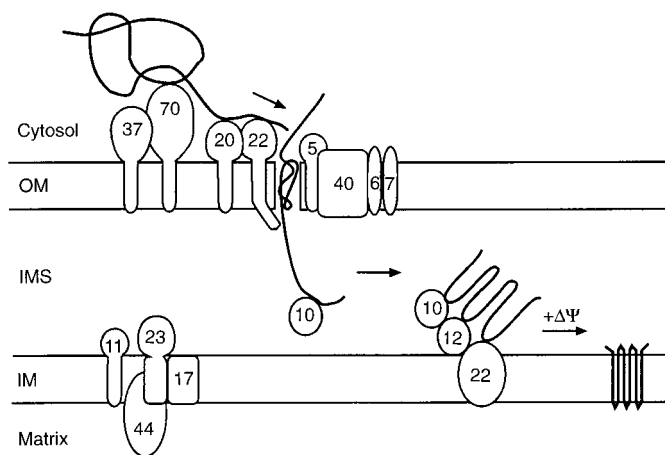
principle only be needed to maintain wild-type Tim22p levels. This model cannot be excluded, but it cannot readily account for our findings that partly imported AAC can be cross-linked to Tim10p in uncoupled wild-type mitochondria and that overexpression of Tim22p fails to suppress loss of Tim10p function. Thus, we favor the view that Tim10p and Tim12p interact directly with each of the different carrier precursors and that Tim22p, because it is a multispinning inner membrane protein, uses this same import pathway.

Proteins similar to Tim10p and Tim12p are present in *Schizosaccharomyces pombe* and *Caenorhabditis elegans* (10), suggesting that this second TIM complex has been conserved throughout evolution. Tim10p and Tim12p may act akin to molecular chaperones: Tim10p may prevent aggregation of the precursor in the intermembrane space, and Tim12p may stabilize the precursor in a conformation that permits its en bloc insertion (18) into the inner membrane.

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9. All *S. cerevisiae* strains were derived from strain GA74D (7). For wild-type controls in mitochondrial experiments, results with strains Tim10, Tim12, and GA74-6A were identical. Unless stated otherwise, we used standard growth media and methods for yeast genetics (19). Temperature-sensitive strains were constructed with the use of a low-fidelity polymerase chain reaction (PCR) followed by in vivo recombination (20, 21). *TIM10* and *TIM12* (including 5' and 3' flanking regions) were subcloned into pRS314 (*CEN*, *TRP1*) and amplified in the presence of 1 mM MgCl₂ and 0.1 to 0.2 mM MnCl₂ with the use of PCR primers located 200 nucleotides from either side of the multiple cloning region. The amplified fragments were purified and co-transformed with linearized pRS314 into yeast strains Δ tim12[*TIM12*] and Δ tim10[*TIM10*] (22). Trp⁺ transformants were selected at 25°C and screened for growth at 25° and 37°C on minimal glucose media containing 5-fluoro-orotic acid and the appropriate growth supplements.
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13. Mitochondria were purified from lactate-grown yeast cells (23) and assayed for in vitro protein import as

Fig. 4. Model for the import of mitochondrial carrier proteins. Proteins 5, 6, 7, 20, 22, 37, 40, and 70 are members of the TOM complex. Proteins 11, 17, 23, and 44 are members of (or closely adjacent to) the Tim23p-Tim17p complex that mediates import of precursors carrying a cleavable NH₂-terminal matrix-targeting sequence. Proteins 10, 12, and 22 are members of the Tim22 complex that mediates import of multispinning carrier proteins into the inner membrane. A carrier precursor exiting the TOM channel is captured by Tim10p in the intermembrane space and delivered to Tim12p, which is bound to Tim22p at the outer face of the inner membrane. Transfer to Tim12p triggers the Tim22p-dependent insertion of the multispinning carrier into the inner membrane.



described (24). The inner membrane potential was abolished with 25 μ M carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP). Nonimported precursor was removed by digestion with proteinase K (200 μ g per milliliter of import buffer) for 15 min at 4°C, followed by addition of 1 mM phenylmethylsulfonyl fluoride (PMSF). Proper insertion of radioactive precursors into the inner membrane was verified by checking resistance to extraction by 100 mM Na₂CO₃ (25) and resistance to proteinase K in mitochondria (26).

14. For cross-linking, *in vitro* import was for 10 min at 20°C, and nonimported precursor was digested with proteinase K (30 μ g/ml). Mitochondria were washed, resuspended at 1 mg/ml in import buffer, and incubated with 1 mM dithiobis(succinimidylpropionate) (DSP) for 30 min at 4°C followed by a quench with 100 mM tris-base, pH 8.0. For immunoprecipitation, solubilized mitochondria were incubated with monospecific polyclonal antibodies coupled to protein A-Sepharose (27).
15. For the multicopy suppressor screen, a yeast genomic DNA library in a 2 μ ,*URA3* vector was transformed into the temperature-sensitive yeast strains tim10-1 and tim12-1 (28). Transformants were grown at 25°C for 24 hours and then shifted to 37°C. Of 5 \times 10⁵ transformants in strain tim12-1, 300 transformants grew at 37°C. Subcloning of the genomic fragment carried by the suppressing plasmid S12 identified *TIM22* as the extragenic suppressor for the *tim12-1* mutation. Of 5 \times 10⁵ transformants in strain tim10-1, 46 transformants grew at 37°C; all of them contained only *TIM10* as the "suppressor" gene.
16. A hexahistidine tag was added to the COOH terminus of Tim10p by PCR amplification of *TIM10* with a primer that inserted six histidine codons immediately upstream of the stop codon. The PCR fragment was subcloned into YCplac111 (creating pTIM10H6) and then sequenced. Strain Tim10H6 was constructed by transformation of strain Δ tim10[*TIM10*] with pTIM10H6 and subsequent removal of the plasmid carrying the wild-type *TIM10* gene on media containing 5-fluoro-orotic acid. To purify Tim10H6p and associated proteins, we solubilized mitochondrial protein (2 mg/ml) in 0.5% digitonin and 20 mM imidazole and incubated it with Ni²⁺-nitrilotriacetic acid-Sepharose (Qiagen, Hilden, Germany). Polyclonal antibodies against Tim10p or Tim12p were raised against the recombinant proteins Tim10p that had been cleaved from a glutathione-S-transferase-Tim10p fusion or a thioredoxin-Tim12p fusion protein, respectively. Polyclonal antibodies against Tim22p were raised against a thioredoxin-Tim22p fusion protein in which the first 16 amino acids of Tim22p had been inserted between amino acids 34 and 35 of thioredoxin.
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***Helicobacter pylori* Adhesin Binding Fucosylated Histo-Blood Group Antigens Revealed by Retagging**

Dag Ilver,* Anna Arnqvist,* Johan Ögren, Inga-Maria Frick, Dangeruta Kersulyte, Engin T. Incecik,† Douglas E. Berg, Antonello Covacci, Lars Engstrand,‡ Thomas Borén§

The bacterium *Helicobacter pylori* is the causative agent for peptic ulcer disease. Bacterial adherence to the human gastric epithelial lining is mediated by the fucosylated Lewis b (Le^b) histo-blood group antigen. The Le^b-binding adhesin, BabA, was purified by receptor activity-directed affinity tagging. The bacterial Le^b-binding phenotype was associated with the presence of the *cag* pathogenicity island among clinical isolates of *H. pylori*. A vaccine strategy based on the BabA adhesin might serve as a means to target the virulent type I strains of *H. pylori*.

Helicobacter pylori, a human-specific gastric pathogen, was first isolated in 1982 (1) and has emerged as the causative agent of chronic active gastritis and peptic ulcer disease (2). Most infected individuals show no clinical symptoms, implicating additional factors, such as genetic predisposition and the genotype of the infecting strain, in disease pathogenesis. Chronic infection is associated with the development of gastric adenocarcinoma, one of the most common types of cancer in humans (3), and *H. pylori* was recently defined as a class I carcinogen (4).

The bacterium colonizes the human gastric mucosa by adhering to the mucous epithelial cells and the mucus layer lining the gastric epithelium (5). These adherence

properties protect the bacteria from the extreme acidity of the gastric lumen and displacement from the stomach by forces such as those generated by peristalsis and gastric emptying. The fucosylated blood group antigens Lewis b (Le^b) and H-1 (Fig. 1A) mediate adherence of *H. pylori* to human gastric epithelial cells *in situ* (6).

We have now biochemically characterized and identified the *H. pylori* blood group antigen-binding adhesin, BabA. Various strains of *H. pylori* were analyzed for binding to ¹²⁵I-labeled fucosylated blood group antigens (Fig. 1B) (7, 8). Three of the five strains examined bound Le^b and H-1. The receptor specificities of these strains for the soluble blood group antigens correlate with their adherence properties *in situ* (6). The prevalence of blood group antigen-binding (BAB) activity was also assessed among 95 recent clinical isolates of *H. pylori*, and 66% (63 isolates) bound the Le^b antigen (7). None of the reference strains or the 95 recent isolates bound to the related Le^a, H-2, Le^x, or Le^y antigens (Fig. 1, A and B). These results support previous observations of the receptor specificity of *H. pylori* for the Le^b and H-1 blood group antigens (6) and, in addition, demonstrate the high prevalence of BAB activity among clinical isolates.

Isolates of *H. pylori* are thought to differ in virulence and those from individuals with peptic ulcers most often are type I strains that express the vacuolating cytotoxin A (VacA) and the cytotoxin-associated gene A (CagA) protein (9). By definition, type II strains express neither marker. Twenty-one strains of previously defined type (10) and 73

D. Ilver, Department of Microbiology, Umeå University, SE-901 87 Umeå, Sweden.

A. Arnqvist, Department of Microbiology and Department of Oral Biology, Umeå University, SE-901 87 Umeå, Sweden.

J. Ögren and T. Borén, Department of Oral Biology, Umeå University, SE-901 87 Umeå, Sweden.

I.-M. Frick, Department of Cell and Molecular Biology, Lund University, SE-221 00 Lund, Sweden.

D. Kersulyte, E. T. Incecik, D. E. Berg, Department of Molecular Microbiology, Washington University Medical School, St. Louis, MO 63110, USA.

A. Covacci, Immunobiological Research Institute Siena, Via Fiorentina 1, 53100 Siena, Italy.

L. Engstrand, Department of Clinical Microbiology and Cancer Epidemiology, University Hospital, SE-751 85 Uppsala, Sweden.

*These authors contributed equally to this work.

†Present address: Max-von Pettenkofer Institut für Hygiene und Medizinische Mikrobiologie, Pettenkoferstrasse 9A, D-81677 Munich, Germany.

‡Present address: Swedish Institute for Infectious Disease Control, S-105 21, Stockholm, Sweden.

§To whom correspondence should be addressed. E-mail: thomas.boren@micro.umu.se