Differential Recognition of Preproteins by the Purified Cytosolic Domains of the Mitochondrial Import Receptors Tom20, Tom22, and Tom70*

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The preprotein translocase of the outer mitochondrial membrane (Tom) is a multi-subunit complex required for specific recognition and membrane translocation of nuclear-encoded preproteins. We have expressed and purified the cytosolic domains of three postulated import receptors, Tom20, Tom22, and Tom70. Each receptor domain is able to bind mitochondrial preproteins but with different specificity. Tom20 binds both preproteins with N-terminal presequences and preproteins with internal targeting signals; the binding is enhanced by the addition of salt. Tom22 selectively recognizes presequence-carrying preproteins in a salt-sensitive manner. Tom70 preferentially binds preproteins with internal targeting information. A chemically synthesized presequence peptide competes with preproteins for binding to Tom20 and Tom22 but not to Tom70. We conclude that each of the three import receptors binds preproteins independently and by a different mechanism. Both Tom20 and Tom22 function as presequence receptors.

Nuclear-encoded mitochondrial proteins are synthesized as preproteins in the cytosol. They are targeted to receptors on the mitochondrial surface and are subsequently translocated across the outer mitochondrial membrane by a general import pore (1–5). The import receptors and the general import pore assemble into a dynamic high molecular weight complex, termed the preprotein translocase of the outer mitochondrial membrane (Tom) (6). Nine Tom proteins have been identified to date. Five of them expose major portions on the cytosolic side of the outer membrane: Tom20-Tom22 and Tom70-Tom37 were reported to be required for import of noncleavable preproteins, carrying (poorly characterized) targeting information in the mature protein but also for cleavable preproteins (8, 23–26). Little is known about the function and specificity of individual receptor proteins. Only the cytosolic domain of Tom70 has been purified so far and used for analyzing binding of preproteins. Schlossmann et al. (27) showed that the cytosolic domain of Tom70 was able to bind noncleavable preproteins but also to some cleavable ones. Schleiff et al. (28) expressed Tom20 as part of a fusion protein with glutathione S-transferase and reported the binding of various preproteins to the fusion protein but also a high degree of nonspecific binding to the beads used.

For this report, we purified and characterized the cytosolic domains of Tom20, Tom22, and Tom70 and directly compared the ability of the three receptors to bind preproteins. We observed that each receptor domain could recognize and bind mitochondrial preproteins independently and with a different specificity. Only with Tom20 and Tom22, a synthetic presequence peptide competed for the binding of preproteins. Our studies indicate that each of the receptors binds mitochondrial preproteins by a different mechanism and show that two distinct presequence receptors function on the mitochondrial surface.

MATERIALS AND METHODS

Construction and Expression of the Cytosolic Domains of Tom20, Tom22, and Tom70—(His)6-tagged cytosolic domains of Tom20, Tom22 and Tom70 were amplified using the polymerase chain reaction and Saccharomyces cerevisiae genomic DNA as template. To amplify yTom20, yHis6 (N-terminal His6 tag), the primers 5′-TATCATATG-GA/GATTTGGAAGAAGATAGCCGGCAATC-3′ and 5′-TATGGATCC-TGATCATCAGGTAGATGATGATGATGATGATGATCTTCCAGC-3′ were used. To amplify TOM70, yHis6 (N-terminal His6 tag), the primers 5′-GTGACATAT-GCGAGAAAAAGAACACGATC-3′ and 5′-GGATCCCTCGAGTTAGATGATGATGATGATGATGATGATGATCTTCCAGC-3′ were used.

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resuspension buffer (10 mM MOPS/KOH, pH 7.2) and aliquoted. After centrifugation and removal of the supernatant, the bacterial pellets were frozen in liquid nitrogen and stored at −80 °C.

**Purification of the Cytosolic Domains by Ni-NTA Affinity Chromatography**—The bacterial pellet was resuspended in binding buffer (5 mM imidazole, 500 mM NaCl, 20 mM Tris/HCl, pH 7.9, containing 2 mM phenylmethylsulfonyl fluoride) and sonified in a Branson sonifier at 50% duty and setting 5 with a microtip by 3 × 10 pulses of 1 s at 0 °C. The suspension was centrifuged at 16,000 × g and 2 °C. The supernatant was applied on 1-ml Mobicol columns (Mobitec) that had been loaded with 800 μl of Ni-NTA-agarose resin slurry (Qiagen) and equilibrated with binding buffer. After an immediate spin in a microcentrifuge, the columns were washed five times with 500 μl of washing buffer (80 mM imidazole, 500 mM NaCl, 20 mM Tris/HCl, pH 7.9). The protein-loaded resin was resuspended in washing buffer and aliquoted.

One aliquot was analyzed by SDS-PAGE to assay the purity and the amount of protein bound to the Ni-NTA-agarose. The identity of the purified proteins was confirmed by Western blotting with the corresponding assay buffer without BSA. Finally all bound proteins were eluted with elution buffer (1M imidazole, 500 mM NaCl, 20 mM Tris/HCl; pH 50% duty) and setting 5 with a microtip by 3 × 10 pulses of 1 s at 0 °C.

One aliquot was analyzed by SDS-PAGE to assay the purity and the amount of protein bound to the Ni-NTA-agarose. The identity of the purified proteins was confirmed by Western blotting with the corresponding antibodies.

**Assay for Binding of Preproteins**—The protein-loaded Ni-NTA resin (final amount of 50 pmol of each Tom protein/100-μl assay) was equilibrated with assay buffer (20 mM imidazole, 0–200 mM KCl, 10 mM MOPS/KOH, pH 7.2, 1% (v/v) BSA) by mixing, centrifugation, and discarding the supernatant (three times). The resuspended resin was divided into equal volumes and transferred into 1-ml Mobicol columns that were closed at the bottom side. A mixture of [35S]-labeled preproteins in assay buffer (maximum 7% (v/v) rabbit reticulocyte lysate) was added, and the resin was resuspended by vortexing. Where indicated, the synthetic presequence peptidase CoxIV(1–23) or the control peptide SynB2, which were blocked N-terminally by acetylation and C-terminally by amidation, were included (to avoid peptide dimers, the cysteine at position 19 of CoxIV(1–23) was replaced by a serine). The total volume of the reaction was 100 μl. The columns were incubated under mixing at 30 °C for 40 min in a thermomixer (Eppendorf, setting 11). After centrifugation, the resin was washed three times with the corresponding assay buffer without BSA. Finally all bound proteins were eluted with elution buffer (1 mM imidazole, 500 mM NaCl, 20 mM Tris/HCl, pH 7.9) and precipitated by adding 12% (v/v) trichloroacetic acid. After 20 min of incubation at 0 °C, the proteins were sedimented by centrifugation (40,000 × g) and washed with acetone. The protein complexes were analyzed by SDS-PAGE and digital autoradiography (phosphor storage imaging technology; Molecular Dynamics).

**Miscellaneous Procedures**—The following procedures were carried out as described previously: synthesis of preproteins in rabbit reticulocyte lysate and labeling with [35S]methionine/[35S]cysteine (29, 30) and centrifugation (40,000 × g); SDS-PAGE (31).

**RESULTS**

**The Purified Domains of Tom20, Tom22, and Tom70 Selectively Bind Mitochondrial Preproteins**—For expression of the cytosolic domains of *S. cerevisiae* Tom20, Tom22 and Tom70, the predicted single membrane anchor sequence of each protein was replaced by a His10 tag. Tom20 and Tom70 carry the predicted single membrane anchor sequence of each protein.

**Expression and purification of the cytosolic domains of Tom20, Tom22, and Tom70.** A, predicted topology of Tom20, Tom22, and Tom70. C terminus; IMS, intermembrane space; N, N terminus; OM, outer mitochondrial membrane. B, expression in *E. coli*. Expression of the recombinant cytosolic domains, residues 30–183 of Tom20, residues 1–97 of Tom22, and residues 38–617 of Tom70 were purified by Ni-NTA chromatography. The labeled preproteins were incubated with the resin-bound cytosolic domains, residues 30–183 of Tom20, residues 1–97 of Tom22, and residues 38–617 of Tom70, which are marked with arrowheads in lanes 2, 3, and 4, respectively. C, purified cytosolic domains. The recombinant proteins were purified by Ni-NTA chromatography. The eluate was analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue R250. The electrophoretic mobility of Tom20cd and Tom22cd was slower than that predicted from the primary structure.

I–3). The control sample contained Ni-NTA resin that was subjected to a mock treatment with lysates from noninduced *E. coli* cells (Fig. 2, lane 4). The following preproteins were synthesized in rabbit reticulocyte lysate in the presence of [35S]methionine/[35S]cysteine: a fusion protein between the presequence of F4-ATPase subunit 9 and entire dihydrofolate reductase (Su9-DHFR) (33); as control, the DHFR (a cytosolic enzyme) alone; a fusion protein containing the presequence plus 87 N-terminal amino acid residues of mature cytochrome b2 and the DHFR (b2DHFR; a 19-residue hydrophobic sorting segment of the 80-residue presequence was deleted) (34); the authentic precursor of cytochrome b2, that includes an N-terminal presequence with a hydrophobic sorting signal and a membrane anchor segment in the mature protein part; the phosphate carrier (P,C) that is synthesized without a presequence but contains internal targeting and membrane anchor sequences. The labeled preproteins were incubated with the resin-bound cytosolic domains at 30 °C under continuous mixing. After washing, bound proteins were eluted and analyzed by SDS-PAGE and digital autoradiography (Fig. 2).
Differential Effect of Salt on Preprotein Binding to Tom22 and Tom20—

The cytosolic domain of Tom22 contains a large number of negatively charged amino acid residues and was suggested to interact with the positively charged amphipathic presequences (7, 9, 10, 32). We thus wondered why Su9-DHFR bound to Tom22cd only with such a low efficiency. The binding assays reported in Fig. 2 were performed in the presence of 100 mM KCl that could interfere with a possible ionic interaction between the charged residues of presequences and Tom22. At low salt, the binding of Su9-DHFR to Tom22cd was indeed strongly enhanced (Fig. 3). Also the binding of b2Δ-DHFR to Tom22cd was increased at low salt, whereas that of cytochrome c1 was largely salt-insensitive, and PγC did not bind to Tom22cd at any salt concentration tested (Fig. 3).

Tom20 contains a segment with negatively charged residues and was similarly suggested to bind preproteins by ionic interactions (7, 10, 21, 35). We asked if lowering of the salt concentration enhanced preprotein binding to Tom20cd. Surprisingly, the binding of Su9-DHFR and b2Δ-DHFR as well as that of cytochrome c1 and PγC to Tom20cd were inhibited at low salt (Fig. 4, lane 1). An increase in the concentration of KCl to 200 mM even enhanced binding of all four preproteins to Tom20cd (Fig. 4, lane 3), whereas DHFR did not bind at any salt concentration tested (Fig. 4, lanes 1–3). The binding of preproteins to Tom20cd and Tom22cd therefore reveals an opposite dependence on the salt concentration (Fig. 4, lanes 1–3 versus 4–6).

Binding of cytochrome c1 and PγC to Tom70cd, however, were only slightly influenced by a variation of the KCl concentration from 0–200 mM (Fig. 4, lanes 7–9). Moreover, Su9-DHFR and b2Δ-DHFR did not bind to Tom70cd at any salt concentration.

We conclude that binding of preproteins to Tom20cd is stimulated by salt, whereas the binding of preproteins to Tom22cd is inhibited by salt. Binding of preproteins to Tom70cd is salt-insensitive. Only Tom20cd is able to bind significant amounts of all four mitochondrial preproteins tested.

A Chemically Synthesized Presequence Peptide Competes with Binding of Preproteins to Tom22 and Tom20—

To further determine the specificity of preprotein binding to the cytosolic domains, we used two chemically synthesized peptides (Fig. 5A). The 23 N-terminal residues of the 25-residue presequence of subunit IV of cytochrome c oxidase (CoxIV) function as a mitochondrial import signal; they can form an amphipathic α-helix with a positively charged side and a hydrophobic side (36–39). The peptide SynB2 contains the same number of pos-

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**Fig. 2.** Differential binding of mitochondrial preproteins to purified receptor domains. Equimolar amounts of the recombinant His-tagged cytosolic domains of Tom20, Tom22, and Tom70 were bound to Ni-NTA columns. For the control, the lysate of the E. coli host strain was subjected to a mock treatment including incubation with Ni-NTA. Then an incubation with 35S-labeled preproteins in assay buffer (with 100 mM KCl) was performed at 30 °C for 40 min as described under “Materials and Methods.” After removal of excess BSA from the columns, the bound protein complexes were eluted and analyzed by SDS-PAGE. Standard (STD) was 5% of labeled preprotein mix that was subjected to a mock treatment including incubation with Ni-NTA. Each of the cytosolic domains interacted with a distinct subset of the proteins added. Tom20cd bound all four mitochondrial preproteins (Fig. 2, lane 1). Tom22cd bound preferentially b2Δ-DHFR and cytochrome c1 and bound only very weakly Su9-DHFR (Fig. 2, lane 2), whereas binding of PγC was in the background range (Fig. 2, lanes 2 and 4). Tom70cd bound cytochrome c1 and PγC but not the DHFR fusion proteins (Fig. 2, lane 3). DHFR alone did not bind to any of the three cytosolic domains (Fig. 2). To minimize unspecific binding of preproteins to the resin-bound cytosolic domains, we included a 300-fold molar excess of BSA (over the purified cytosolic domains) in the binding assay; however, the omission of BSA did not affect the efficiency and selectivity of preprotein binding (not shown). We conclude that the cytosolic domains of Tom20, Tom22, and Tom70 selectively bind distinct subsets of mitochondrial preproteins.

**Fig. 3.** Binding of preproteins to the cytosolic domain of Tom22 is salt-sensitive. The binding assay and the quantification were performed as described in the legend of Fig. 2 except that the concentration of KCl was varied from 0 to 200 mM.
itively charged residues but lacks the hydrophobic site and does not function as mitochondrial import signal (36).

Binding of preproteins to Tom22 cd was performed in the presence of different concentrations of the presequence peptide or the control peptide. The presequence peptide strongly inhibited the binding of Su9-DHFR and b2-D-DHFR (Fig. 5B, lanes 2–7). Half-maximal inhibition of preprotein binding occurred at \( \frac{1}{6} \) mM peptide in the case of Su9-DHFR and at \( \frac{1}{10} \) mM peptide in the case of b2-D-DHFR (Fig. 5C). In concentrations up to 20 mM, the peptide SynB2 revealed only a slight inhibitory effect on binding of these two preproteins to Tom22 cd (Fig. 5B, lanes 8 and 9, and C); at 30 mM, it caused a partial inhibition of binding (Fig. 5B, lane 10, and C). The inhibitory effect of the presequence peptide on binding of cytochrome c1 to Tom22 cd was not as strong as that on the binding of the two DHFR fusion proteins but still significantly distinct from that of the peptide SynB2 (Fig. 7A, lanes 13–27, and C). The inhibitory effect of the presequence peptide on binding of cytochrome c1 to Tom22 cd was not as strong as that on the binding of the two DHFR fusion proteins but still significantly distinct from that of the peptide SynB2 (Fig. 7A, lanes 13–27, and C). Binding of the noncleavable preprotein P C to Tom20 cd, however, was not competed for by the presequence peptide (Fig. 7, A and C).

We conclude that a chemically synthesized presequence peptide competes with the binding of cleavable preproteins to Tom20 cd and Tom22 cd. Binding of preproteins to Tom70 cd is not affected by the presequence peptide.

**DISCUSSION**

We report that the purified cytosolic domains of yeast Tom20, Tom22, and Tom70 selectively bind distinct subsets of mitochondrial preproteins. The overall pattern of preprotein binding to the recombinant domains agrees well with the preprotein specificities that have been predicted for these Tom proteins by studying their function with mitochondria or outer membrane vesicles (summarized in Refs. 1, 3, and 40). Together with the lack of binding of a nonmitochondrial protein and the competition by a synthetic presequence peptide, this
underscores the validity of the in vitro binding assays presented here.

The analysis in the purified system provides several new insights in the mechanism of action of mitochondrial import receptors. First, each cytosolic domain alone can specifically bind preproteins without a requirement for other Tom proteins. Second, the characteristics of binding of the same preprotein are different for each of the cytosolic domains, suggesting different functional mechanisms for the three import receptors. Although it has been expected that Tom70, which preferentially binds preproteins with internal targeting information (23–27), functions differently than Tom20 and Tom22, the latter two receptors were assumed to bind preproteins by a similar mechanism. Both were suggested to bind positively charged presequences in a salt-sensitive (ionic) manner (7, 9, 10, 21, 35). Moreover, Mayer et al. (10) proposed that Tom20 and Tom22 do not constitute independent binding sites for preproteins but function as a single heterodimeric receptor.

The findings reported here suggest the following characteristics of mitochondrial import receptors. (i) Tom22: Binding to Tom22cd requires the presence of a cleavable presequence. Binding of a preprotein, which consists only of an amphipathic mitochondrial presequence and a cytosolic passenger protein, is highly sensitive to the addition of salt and is strongly competed for by a synthetic presequence peptide. This suggests that binding occurs solely via the presequence and that the positively charged surface of the presequence interacts with the highly negatively charged Tom22cd (32) in an ionic manner. (ii) Tom20: Cleavable and noncleavable preproteins bind to yeast Tom20cd, indicating a broad substrate specificity for this import receptor in agreement with the binding properties of a fusion protein between glutathione S-transferase and human Tom20 (28). The binding of preproteins to yeast Tom20cd is stimulated by the addition of salt, leading to the unexpected conclusion that preprotein recognition by Tom20cd is not mediated by ionic forces but probably by a hydrophobic type of interaction. Tom20cd probably interacts with the hydrophobic surface of the amphipathic presequences. Interestingly, a higher concentration of a control peptide, which contains the same number of positive charges as the presequence peptide but lacks the hydrophobic character, partially inhibits preprotein binding to Tom22cd but not to Tom20cd. The opposite influence of salt supports the view that Tom22cd and Tom20cd recognize distinct surfaces of a presequence. It has been shown that synthetic presequences form amphipathic \(\alpha\)-helices at amphipathic surfaces such as detergent micelles or lipid bilayers but not in water (37–39), suggesting that the interaction of presequences with the binding site/cleft of a receptor may induce the formation of an \(\alpha\)-helical structure. Because a synthetic presequence peptide competes with the binding of cleavable preproteins but not with that of a noncleavable one to Tom20cd, it is likely that Tom20 employs distinct mechanisms for recognition of presequences and internal targeting sequences. (iii) Tom70: Binding of preproteins to Tom70cd is largely insensitive to the presence or the absence of salt and is not competed for by the presequence peptide, clearly distinguishing the binding properties from that of Tom22cd and Tom20cd. Tom70cd apparently does not recognize mitochondrial presequences but targeting information contained in the mature part of preproteins.

The cleavable preprotein cytochrome \(c_1\) behaves differently than other cleavable preproteins because it does not only bind to Tom20cd and Tom22cd but also to Tom70cd. This broad receptor dependence of cytochrome \(c_1\) is in agreement with import studies of this preprotein into mitochondria (26, 41). Moreover, the binding to Tom20cd and Tom22cd is only partially competed for by the synthetic presequence peptide, and the binding to Tom22cd is only slightly inhibited by increasing the salt concentration. This suggests that cytochrome \(c_1\) may not only carry targeting information within the amphipathic part.
of the presequence but also in additional protein parts. Although further work is needed to identify putative internal targeting signals of cytochrome c₁, previous studies on the import of chimeric preproteins into mitochondria indeed suggested that some cleavable preproteins carry targeting information not only in the presequence but also within the mature protein (42–44).

The binding characteristics observed with different preproteins raise the speculation that Tom20<sub>cd</sub> as well as Tom22<sub>cd</sub> may contain more than a single binding site for preproteins each. (i) The major binding site of Tom22<sub>cd</sub> is obviously that for the positively charged surface of presequences. In addition, internal targeting information of a preprotein can stabilize the binding (cytochrome c₁) but is not sufficient to promote binding by itself (P.C). A putative second binding site on Tom22<sub>cd</sub> would thus not function independently but would play an auxiliary role. (ii) Tom20<sub>cd</sub> may contain two binding sites, one for the hydrophobic surface of presequences and one for internal targeting information, each of which is apparently competent for recognition of preproteins. (iii) Tom70<sub>cd</sub> contains a binding site for internal targeting information that is not influenced by presequences.

Irrespective of these speculations, it is evident that each of the three import receptors Tom20, Tom22, and Tom70 can bind mitochondrially preproteins independently of other Tom proteins. In particular, this demonstrates that two presequence receptors operate at the mitochondrial outer membrane. The concentration of presequence peptide needed for half-maximal binding (4–10 µM) suggests a relatively low affinity for the import receptors. The presence of two presequence receptors that recognize distinct properties of the same presequence should thus strongly enhance the specificity and fidelity of preprotein recognition at the mitochondrial surface.

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REFERENCES

1. Kubrich, M., Dietmeier, K., and Pfanner, N. (1995) Curr. Genet. 27, 393–403
2. Ryan, K. R., and Jensen, R. E. (1995) Cell 83, 517–519
3. Lill, R., and Neupert, W. (1996) Trends Cell. Biol. 6, 56–61
4. Schatz, G., and Dobberstein, B. (1996) Science 271, 1519–1526
5. Pfanner, N., and Meijer, M. (1997) Curr. Biol. 7, R100–R103
6. Pfanner, N., Douglas, M. G., Endo, T., Hosgrenrad, N. J., Jensen, R. E., Meijer, M., Neupert, W., Schatz, G., Schmitz, U. K., and Shore, G. C. (1996) Trends Biochem. Sci. 21, 51–52
7. Bolliger, L., Junne, T., Schatz, G., and Lithgow, T. (1995) EMBO J. 14, 6318–6326
8. Gratzer, S., Lithgow, T., Bauer, R. E., Lamping, E., Paltauf, F., Kohlwein, S. D., Hauke, V., Junne, T., Schatz, G., and Horst, M. (1995) J. Cell. Biol. 129, 25–34
9. Henlinger, A., Kubrich, M., Moczkö, M., Gartner, F., Mallet, L., Bussemer, F., Eckerskorn, C., Lottspeich, F., Dietmeier, K., Jacquet, M., and Pfanner, N. (1995) Mol. Cell. Biol. 15, 3382–3389
10. Mayer, A., Nargang, F. E., Neupert, W., and Lill, R. (1995) EMBO J. 14, 4204–4211
11. Bömer, U., Pfanner, N., and Dietmeier, K. (1996) FEBS Lett. 382, 153–158
12. Schlossmann, J., Lill, R., Neupert, W., and Court, D. A. (1996) J. Biol. Chem. 271, 17890–17895
13. Vestweber, D., Brunner, J., Baker, A., and Schatz, G. (1989) Nature 341, 205–209
14. Kiebler, M., Pfäffler, R., Söllner, T., Griffiths, G., Horstmann, H., Pfanner, N., and Neupert, W. (1990) Nature 348, 610–616
15. Dietmeier, K., Henlinger, A., Bömer, U., Dekker, P. J. T., Eckerskorn, C., Lottspeich, F., Kubrich, M., and Pfanner, N. (1997) Nature, in press
16. Alenodra, A., Kubrich, M., Moczkö, M., Henlinger, A., and Pfanner, N. (1995) Mol. Cell. Biol. 15, 6196–6205
17. Henlinger, A., Bömer, U., Alenodra, A., Eckerskorn, C., Lottspeich, F., Dietmeier, K., and Pfanner, N. (1996) EMBO J. 15, 2125–2137
18. Söllner, T., Griffiths, G., Pfäffler, R., Pfanner, N., and Neupert, W. (1989) Cell 59, 1061–1070
19. Schneider, H., Söllner, T., Dietmeier, K., Eckerskorn, C., Lottspeich, F., Trulzsch, B., Neupert, W., and Pfanner, N. (1991) Science 254, 1659–1662
20. Ramage, L., Junne, T., Hahne, K., Lithgow, T., and Schatz, G. (1995) EMBO J. 14, 4115–4123
21. Moczkö, M., Khann, M., Gartner, F., Henlinger, A., Schafer, E., and Pfanner, N. (1994) J. Biol. Chem. 269, 9045–9051
22. Lithgow, T., Junne, T., Soza, K., Gratzer, S., and Pfanner, N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11973–11977
23. Söllner, T., Pfäffler, R., Griffiths, G., Pfanner, N., and Neupert, W. (1990) Cell 62, 107–115
24. Steger, H. F., Söllner, T., Kiebler, M., Dietmeier, K. A., Pfäffler, R., Trulzsch, K. S., Tropschug, M., Neupert, W., and Pfanner, N. (1990) J. Cell Biol. 111, 2353–2363
25. Hines, V., Brands, A., Griffiths, G., Horstmann, H., Brutsch, H., and Schatz, G. (1996) EMBO J. 15, 3191–3200
26. Hines, V., and Schatz, G. (1993) J. Biol. Chem. 268, 449–454
27. Schlossmann, J., Dietmeier, K., Pfanner, N., and Neupert, W. (1994) J. Biol. Chem. 269, 11893–11891
28. Schlieff, K., Shore, G. C., and Goping, I. S. (1997) FEBS Lett. 404, 314–318
29. Pelham, H. R. B., and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247–256
30. Alenodra, A., Gartner, F., Henlinger, A., Kubrich, M., and Pfanner, N. (1995) Methods Enzymol. 269, 363–386
31. Laemmli, U. K. (1970) Nature 227, 680–685
32. Kiebler, M., Keil, P., Schneider, H., van der Klei, I. J., Pfanner, N., and Neupert, W. (1993) Cell 74, 483–492
33. Pfanner, N., Tropschug, M., and Neupert, W. (1997) Cell 90, 815–823
34. Koll, H., Gaul, B., Rassow, J., Ostermann, J., Horwich, A. L., Neupert, W., and Hartl, F. U. (1992) Cell 68, 1163–1175
35. Hauke, V., Lithgow, T., Rospert, S., Hahne, K., and Schatz, G. (1995) J. Biol. Chem. 270, 5565–5570
36. Allison, D. S., and Schatz, G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9011–9015
37. Roise, D., Horvath, S. J., Tomich, J. M., Richards, J. H., and Schatz, G. (1986) EMBO J. 5, 1327–1334
38. Roise, D., Theiler, F., Horvath, S. J., Tomich, J. M., Richards, J. H., Allison, D. S., and Schatz, G. (1988) EMBO J. 7, 649–653
39. Tam, L. K. and Bartoldus, I. (1990) FEBS Lett. 272, 29–33
40. Lithgow, T., Glick, B. S., and Schatz, G. (1995) Trends Biochem. Sci. 20, 98–101
41. Henlinger, A., Keil, P., Nelson, R. J., Craig, E. A., and Pfanner, N. (1995) Biol. Chem. 376, 515–519
42. Bedwell, D. M., Klionsky, D. J., and Emr, S. D. (1987) Mol. Cell. Biol. 7, 4038–4047
43. Pfanner, N., Muller, H. K., Harmey, M. A., and Neupert, W. (1987) EMBO J. 6, 3449–3454
44. Zara, V., Palmieri, F., Mahlke, K., and Pfanner, N. (1992) J. Biol. Chem. 267, 12077–12081
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