N-terminal Hydrophobic Sorting Signals of Preproteins Confer Mitochondrial hsp70 Independence for Import into Mitochondria*

(Received for publication, March 28, 1997, and in revised form, May 13, 1997)

Albrecht Gruhler, Isabel Arnold, Tilman Seytter, Bernard Guiard, Elisabeth Schwarz, Walter Neupert, and Rosemary A. Stuart‡

From the Institut für Physiologische Chemie, Universität München, Goethestraße 33, 80336 München, Federal Republic of Germany

The requirement of mitochondrial hsp70 (mt-hsp70) for the import of a series of preproteins containing hydrophobic sorting signals into isolated yeast mitochondria was investigated. Here we demonstrate that the presence of such a sorting signal in proximity to the N-terminal matrix-targeting sequence of a preprotein can secure a translocating polypeptide chain in the import channel in a manner that does not require mt-hsp70 activity. Trapping the translocating chain in this fashion leads to efficient processing by the mitochondrial processing peptidase and to complete translocation across the outer mitochondrial membrane into the intermembrane space. These mt-hsp70-independent effects appear to be exerted at the level of the inner membrane through an interaction of the hydrophobic core of the sorting signal with component(s) of the translocase of the inner membrane. Hydrophobic sorting signals of inner membrane proteins inserted into the membrane from the matrix, as well as those of intermembrane space proteins, are capable of causing this mt-hsp70-independent stabilization, demonstrating that this phenomenon is not unique to those preproteins normally sorted to the intermembrane space.

The import of nuclear encoded preproteins into the mitochondrial matrix involves the coordinated action of translocation machineries in both the outer and inner mitochondrial membranes (1–3). The majority of these preproteins are targeted to the mitochondria by N-terminal targeting sequences that are processed in the matrix by the mitochondrial processing peptidase (MPP)1 (4, 5). In addition to their targeting function, presequences initiate translocation across the inner membrane via the TIM (translocase of the inner membrane) machinery in a Δψ-dependent manner (6–12). Binding of the molecular chaperone mt-hsp70, together with TIM44, a component of the TIM machinery, serves to hold the presequence upon its exposure to the matrix and thus confers unidirectionality on the import process (13, 14). Studies with matrix-targeted preproteins suggested that binding of mt-hsp70 to the preprotein is the only or major force for achieving vectorial movement at the initial steps of import. Supporting this, retrograde movement of the translocating polypeptide was observed if bound mt-hsp70 was released (12, 15, 16). On the other hand, the activity of mt-hsp70 was observed not to be required to initiate translocation of some preproteins targeted to the inner membrane (17–20).

To study the mechanism by which mt-hsp70 function is bypassed, we analyzed the import of preproteins either destined for the inner membrane (which are first imported into the matrix; see below) or targeted to the intermembrane space. These preproteins all contain hydrophobic sorting signals. Their import in the presence or absence of functional mt-hsp70 was investigated. The precursor proteins chosen consisted of N-terminal segments of either pre-subunit 9 of the F1F0-ATPase or preocytochrome b2 fused to mouse dihydrofolate reductase (DHFR). The pre-subunit 9 chimera included the first transmembrane domain of subunit 9 fused to DHFR (pSu9-(1–112)-DHFR) and attains an Nout-Cin topology across the inner membrane in an export event following initial import into the matrix (20). Cytochrome b2, on the other hand, is targeted to the intermembrane space by a cleavable N-terminal bipartite presequence that is initially processed by MPP. According to recent evidence, the sorting signal targets cytochrome b2 along a sorting pathway that involves at least partial passage through the matrix (21). This import pathway differs, however, from that of matrix-targeted preproteins, as it appears to disengage from mt-hsp70 machinery at an early stage of the import process (22).

In this paper, we report that the hydrophobic sorting signal of a preprotein, when in proximity to a matrix-targeting sequence, can guarantee the maintenance of a translocating polypeptide in the TIM channel in the apparent absence of mt-hsp70 activity, thereby efficient MPP processing, as well as translocation across the outer membrane, takes place under conditions when further translocation across the inner membrane is blocked. Sorting signals of both a matrix-targeted protein and an intermembrane space protein were capable of mediating this mt-hsp70-independent securing of translocating polypeptides in the TIM machinery. The common denominator for maintaining a preprotein in the TIM channel is therefore a suitably positioned hydrophobic core rather than a sorting signal specific for one mitochondrial subcompartment over another. We propose that hydrophobic segments retard the sliding of a translocating chain in the import channel to facilitate recognition and lateral exit of those preproteins that become inserted into the inner membrane.

EXPERIMENTAL PROCEDURES

Isolation of Yeast Mitochondria—Saccharomyces cerevisiae wild-type strain D273-10B was grown in lactate medium (23) at 30 °C, whereas the temperature-sensitive mutant of mt-hsp70, see1-3 (PK83), and its corresponding wild type (PK82) (24) were grown at 24 °C. Mitochondria were isolated as described previously (23, 27).

Precursor Proteins—DNA encoding precursor proteins was transcribed with SP6 RNA polymerase as described previously for the

* This work was supported by Sonderforschungsbereich 184 Teilprojekt B2 and by the Genzentrum München. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 49-89-5996-295; Fax: 49-89-5996-270; E-mail: Stuart@bio.med.uni-muenchen.de.

1 The abbreviations used are: MPP, mitochondrial processing peptidase; mt-hsp70, mitochondrial hsp70; DHFR, dihydrofolate reductase; MOPS, 4-morpholinepropanesulfonic acid; PAGF, polyacrylamide gel electrophoresis.
cytochrome b$_2$-derived precursors (18, 25) and the pSu9-DHFR fusion proteins (15). All precursor proteins were then synthesized in rabbit reticulocyte lysate (Promega) in the presence of [35S]methionine (26).

Import into Mitoplasts and Mitochondria—Isolated mitochondria were converted to mitoplasts prior to import as follows. Mitochondria were diluted 10-fold in 10 mM potassium phosphate buffer and incubated for 20 min at 0 °C prior to resolation and resuspension in SEM buffer (250 mM sucrose, 2 mM EDTA, 10 mM MOPS, pH 7.2).

Import was performed at 25 °C in the following buffer: 220 mM sucrose, 80 mM KCl, 10 mM MOPS/KOH, pH 7.2, 3% bovine serum albumin (w/v) 25 mM potassium phosphate, 5 mM MgOAc, and 1 mM MnCl$_2$. Import mixtures contained 2 mM NADH, 3 mM hemin, 0.3 mM glycerol, 1% (v/v) reticulocyte lysate containing the radiolabeled precursor proteins. Samples were treated with proteinase K (50 μg/ml) for 30 min at 0 °C under either non-swelling or hypotonic swelling conditions (see below) (27). Samples were analyzed by SDS-PAGE and immunoblotting onto nitrocellulose. The efficiency of swelling of the mitochondria was assessed following immunodecoration of the blot with antisera against endogenous cytochrome c peroxidase (soluble intermembrane space protein) and Mge1p (matrix-localized protein). Swelling in each case was >95% efficient, whereas the integrity of the inner membrane was not perturbed (data not shown).

Methotrexate Arrest of DHFR Fusion Proteins—To stall the import of the various DHFR fusion proteins with methotrexate/NADPH, translation of the radiolabeled proteins was performed at 25 °C in the presence of 1 μM methotrexate. The reticulocyte lysates were diluted in import buffer containing 1 μM methotrexate and 1 mM NADPH and incubated for 5 min at 0 °C followed by 5 min at 25 °C prior to the addition of mitochondria.

Depletion of Matrix ATP—Isolated mitochondria were depleted of their free matrix ATP by treatment with oligomycin and carboxyatractyloside as described previously (28). When matrix ATP levels were to be restored in depleted mitochondria following an import reaction, mitochondria were incubated in import buffer with oligomycin (20 μM) and apyrase (40 units/ml) for 3 min at 25 °C, and the carboxyatractyloside was omitted. (This depletion procedure resulted in a >90% import inhibition of the matrix-targeted preprotein pSu9-(1–69)-DHFR (data not shown).) A similar ATP independence of import was obtained for pSu9-(1–112)-DHFR following an import reaction. Mitochondria were isolated wild-type (wt) or ssc1-3 (13-3) mitochondria, which had been preincubated for 10 min at 37 °C. Samples were analyzed by SDS-PAGE and fluorography. p, precursor; i, intermediate; m, mature.

RESULTS

Requirement of mt-hsp70 Activity for the Stable Accumulation of Translocation Intermediates—To study the ability of hydrophobic sequences in preproteins to interact with the TIM translocation channel, the import of a number of precursor proteins was analyzed: the cytochrome b$_2$-derived preproteins p$_{b_2}$(1–109)-DHFR, p$_{b_2}$(1–85)-DHFR, and p$_{b_2}$(1–109)-DHFR and the matrix-targeted inner membrane protein pSu9-(1–112)-DHFR (Fig. 1A). Import was performed initially in the presence of methotrexate to arrest the DHFR domain in a TIM-anchoring conformation and thereby allow only translocation of the N-terminal region of the precursor across the inner membrane. The positions of the hydrophobic segments in the various preproteins relative to MPP processing sites are depicted in Fig. 1A.

Accumulation of intermediates processed by MPP was used as an indicator for the transfer of the matrix-targeting sequence across the inner membrane into the matrix. When imported into wild-type mitochondria, all precursors arrested with methotrexate were efficiently processed by MPP and were reisolated with the mitochondria (Fig. 1B). Upon import into mitochondria containing inactivated mt-hsp70 from the temperature-sensitive ssc1-3 mutant (24), the processing of p$_{b_2}$(1–76)-DHFR, p$_{b_2}$(1–85)-DHFR, and pSu9-(1–112)-DHFR by MPP was inhibited. In contrast, p$_{b_2}$(1–109)-DHFR was efficiently processed to its intermediate-size form in the absence of mt-hsp70 activity (Fig. 1B). Thus, despite that all three precursors contain hydrophobic segments, only methotrexate-arrested p$_{b_2}$(1–109)-DHFR can be accumulated as an MPP-processed translocation intermediate in intact mitochondria in the absence of functional mt-hsp70. On the other hand, all of these methotrexate-arrested preproteins can be accumulated as MPP-processed translocation intermediates upon import into mitoplasts in the absence of functional mt-hsp70 (data not shown). These findings suggest that the hydrophobic segment must extend at least to the inner membrane to secure stabilization of a translocation intermediate in an mt-hsp70-independent manner.

Matrix ATP Requirement for the Import and Sorting of Preproteins—Depletion of matrix ATP levels impairs the ATP-dependent activity of mt-hsp70 and leads to an almost complete block of import of hydrophilic matrix-targeted proteins (13, 19, 28). The cytochrome b$_2$-derived precursor proteins analyzed were efficiently processed by MPP and were imported to a protease-resistant location in the mitochondria in a matrix ATP-independent manner (Fig. 2, upper panel, +ATP versus −ATP). A similar ATP independence of import was obtained for the pSu9-(1–112)-DHFR protein, despite that it is a matrix-targeted protein (Fig. 2, lower panel, +ATP versus −ATP). This is in marked contrast to the hydrophilic matrix-targeted precursor protein pSu9-(1–69)-DHFR, where import was strictly matrix ATP-dependent (Fig. 2, lower panel). Subfractionation of the mitochondria by hypotonie swelling revealed one important difference between the ATP-containing and ATP-depleted mitochondria. In the presence of matrix ATP, both p$_{b_2}$(1–76)-DHFR (Fig. 2, upper panel, lane 3) and pSu9-(1–112)-DHFR (Fig. 2, lower panel, lane 9) were imported into the mitochondrial matrix, as they were largely protease-protected in mito-
plasts. In ATP-depleted mitochondria, efficient translocation across the outer membrane occurred, but both proteins became arrested in the inner membrane during import in an N<sub>in</sub>-C<sub>out</sub> topology. This is demonstrated by MPP processing of the N-terminal presequence and accessibility of the C-terminal DHFR domain to added protease in mitoplasts (Fig. 2, upper panel, lane 6; and lower panel, lane 12). Therefore, their import into the matrix was arrested at the level of the inner membrane in the absence of matrix ATP, indicating lack of functional mt-hsp70. In the case of the other cytochrome <i>b</i> <sub>2</sub>-derived preproteins, an N<sub>in</sub>-C<sub>out</sub> topology was achieved following import into both matrix ATP-containing and ATP-depleted mitochondria. Thus, the sorting process appears to proceed in a manner independent of functional mt-hsp70 (Fig. 2, d). Thus, the sorting process appears to proceed in a manner independent of functional mt-hsp70 (Fig. 2, d).

Differential Requirement for mt-hsp70 in Preventing Retrograde Movement of Translocation Intermediates—We asked whether the hydrophobic segment in a preprotein is sufficient to prevent retrograde translocation of methotrexate-arrested intermediates when stalled at the level of the inner membrane following the release of bound mt-hsp70. Retrograde translocation of methotrexate-arrested preproteins was analyzed in both mitochondria and mitoplasts (Fig. 3). p<sub>b2</sub>-(1–76)-DHFR, p<sub>b2</sub>-(1–85)-DHFR, and p<sub>b2</sub>-(1–109)-DHFR were imported into either wild-type mitochondria or mitoplasts in the presence of methotrexate, where they became arrested as translocation intermediates, processed by MPP in the matrix (Fig. 3). These translocation intermediates were associated with the import sites in a stable manner when further incubated in the presence of ATP and a membrane potential. Depletion of the matrix ATP results in the release of bound mt-hsp70 from the translocating polypeptide chain (15). Under these conditions, the MPP-processed forms of p<sub>b2</sub>-(1–76)-DHFR and p<sub>b2</sub>-(1–85)-DHFR underwent retrograde translocation and were released from the mitochondria into the supernatant (Fig. 3, +Oli/Ap). Dissipation of the membrane potential by addition of valinomycin led also to a release of the methotrexate-arrested intermediates (Fig. 3, +Val). In contrast, the MPP-processed form of p<sub>b2</sub>-(1–109)-DHFR remained associated with the mitochondria under both of these conditions, suggesting that it had become stabilized in a manner no longer dependent on Δψ and mt-hsp70 binding. When accumulated in mitoplasts, all preproteins remained tightly associated as translocation intermediates, even under conditions of matrix ATP depletion and Δψ dissipation (Fig. 3, hatched bars). A control hydrophilic matrix-targeted protein, p<sub>Su9</sub>-(1–69)-DHFR, when arrested with methotrexate in mitoplasts and mitochondria, underwent retrograde translocation upon both of these treatments (data not shown) (12).

In conclusion, penetration of the hydrophobic segment to the translocase of the inner membrane leads to maintenance in the import channel and prevention of retrograde translocation upon release of bound mt-hsp70. The common denominator for mt-hsp70-independent stabilization appears to be the interaction of a suitably positioned hydrophobic core with component(s) of the TIM channel, rather than a particular recognition of a sorting signal specific for one mitochondrial sublocation or another.

### Matrix ATP Requirement for the Stabilization of Cytochrome b<sub>2</sub> Bearing a Mutation in the Sorting Signal

To investigate the specificity of the sorting signal in more detail, we analyzed the import of a cytochrome <i>b</i> <sub>2</sub> derivative, p<sub>b2</sub><sup>RIC</sup>-(1–167)-DHFR, which bears mutations in the essential cluster of positively charged amino acids (Arg-Lys-Arg) just prior to the hydrophobic core of the sorting signal. This mutated preprotein becomes missorted to the matrix in an ATP/mnt-hsp70-dependent manner. In the absence of functional mt-hsp70, it is imported into the intermembrane space, where it is matured by the Imp1p protease (18). In the presence of matrix ATP, p<sub>b2</sub><sup>RIC</sup>.

### Table: mt-hsp70-independent Import of Proteins into Mitochondria

| Protein          | ATP Requirement | Presence of Matrix ATP | Presence of ATP-depleted Mitochondria |
|------------------|-----------------|------------------------|--------------------------------------|
| p<sub>b2</sub>-(1–76)-DHFR | +                | +                      | +                                    |
| p<sub>b2</sub>-(1–85)-DHFR | +                | +                      | +                                    |
| p<sub>b2</sub>-(1–109)-DHFR | +                | +                      | +                                    |

### Figure 2: Import of precytochrome <i>b</i> <sub>2</sub>-DHFR and p<sub>Su9</sub>-DHFR fusion proteins into matrix ATP-containing or ATP-depleted mitochondria.

Radiolabeled preproteins were imported into matrix ATP-containing (+ATP) or ATP-depleted (−ATP) mitochondria. Following a proteinase K (Prot. K) treatment under either non-swelling or swelling conditions as indicated, samples were analyzed by SDS-PAGE and Western blotting. p, precursor; i, intermediate; m, mature.
(1–167)-DHFR was rapidly imported into the mitochondria (Fig. 4), where it was localized to the matrix (data not shown). The decrease in the signal at later time points reflected degradation of the matrix-accumulated species by the Pim1p protease, as reported previously (31). In ATP-depleted mitochondria, pb2_{RIC}-(1–167)-DHFR was imported with reduced kinetics (Fig. 4), and it accumulated in the intermembrane space (data not shown) (18). On the other hand, wild-type pb2-(1–167)-DHFR was largely unaffected by alterations in the matrix ATP levels (Fig. 4). Despite the mutation in the sorting signal, pb2_{RIC}-(1–167)-DHFR could be as effectively imported into the mitochondria as its wild-type counterpart in the absence of functional mt-hsp70. We conclude therefore that the mt-hsp70-independent stabilization observed is not dependent on a functional sorting signal, but rather on the presence of an intact hydrophobic core.

**DISCUSSION**

The import of mitochondrial preproteins containing N-terminal hydrophobic segments does not depend on an early interaction with mt-hsp70. As we show here, this is in contrast to the import of hydrophilic proteins. By virtue of such hydrophobic segments, preproteins become stabilized in the TIM machinery. This occurs apparently by interaction with component(s) that either are part of the TIM channel or are in close proximity to the TIM channel. Stabilization in this manner can lead to processing by MPP in the matrix and completion of passage over the outer membrane.
Second, the hydrophobic segments must penetrate into the inner membrane to stabilize the translocating polypeptide chain in the import channel in a membrane potential- and an mt-hsp70-independent manner. If the sorting signal is physically restricted from reaching as far as the inner membrane (e.g., by a folded DHFR moiety), both the presence of a membrane potential and binding of mt-hsp70 to the incoming polypeptide are necessary for the prevention of retrograde translocation. The mt-hsp70-independent stabilization may be exerted by an interaction of the hydrophobic core with components of the TIM machinery in the inner membrane or by unknown components in the mitochondrial matrix.

Third, the position of the hydrophobic core relative to the presequence is critical for a preprotein to become stabilized in an mt-hsp70-independent manner. From results presented here and from the analysis of other inner membrane proteins such as cytochrome oxidase subunit Va and Sco1 (13, 32), it appears that a maximum distance of ~75 amino acids from the N terminus of the preprotein to its hydrophobic core can be tolerated if mt-hsp70-independent stabilization is to be ensured. In the case of cytochrome oxidase subunit Va, where the hydrophobic segment is ~100 amino acid residues from the N terminus, import is dependent on mt-hsp70 activity (13, 32); moving the hydrophobic sorting signal of cytochrome oxidase subunit Va closer to the N terminus conferred mt-hsp70 independence on the import process (32).

These findings support the following mechanism of protein transport across the inner membrane. Translocation of polypeptides can occur, at least of the N-terminal ~75 amino acid residues, by random reversible diffusion (33); mt-hsp70, by binding to preproteins upon their emergence in the matrix, then acts to shift the equilibrium to the trans-side of the inner membrane. The free diffusion across the inner membrane of these N-terminal regions would not support an import model where mt-hsp70 functions solely in a manner whereby it pulls segments of the polypeptide chain into the matrix (34, 35).

Our data suggest that matrix-targeted proteins containing a suitably positioned hydrophobic segment can artificially become arrested in the inner membrane. Is such a hydrophobic arrest mechanism used by at least a subset of inner membrane proteins, such as cytochrome oxidase subunit Va, as a physiological targeting pathway? Sorting by hydrophobic arrest most likely exists for some inner membrane proteins. We speculate that the TIM channel, perhaps together with other protein components of the inner membrane, scans the incoming polypeptide for the presence of hydrophobic cores. Recognition of such an element would result in a slowdown in translocation of the preprotein. Such a translocational arrest is necessary if the sorting signal is to laterally insert into the inner membrane. Hydrophilic sequences flanking the hydrophobic core appear to play an important role in the specificity of the process of lateral integration into the inner membrane (4).

If such a lateral sorting signal is not present, translocation will proceed, resulting in matrix import of the hydrophobic core. This further translocation requires active mt-hsp70. If absent, the affinity of component(s) of the TIM channel for such a hydrophobic core is sufficient to trap the incoming polypeptide in the import channel. Under these conditions, complete translocation across the outer membrane can occur, but import across the inner membrane is blocked.

---

8 A. Gruhler, I. Arnold, T. Seytter, B. Guiard, E. Schwarz, W. Neupert, and R. A. Stuart, unpublished results.

3 I. Arnold and R. A. Stuart, unpublished results.

4 E. E. Rojo and R. A. Stuart, manuscript in preparation.
Is this arrest mechanism used by the cytochrome b_{5}-derived proteins? The rates and efficiencies of import of these preproteins were found not to be adversely affected by the lack of mt-hsp70 activity. These data suggest that the cytochrome b_{5}-derived preproteins follow the same sorting route irrespective of whether mt-hsp70 is functional or not. Only in the case of those cytochrome b_{5}-derived proteins bearing an intact folded heme domain is their import dependent on mt-hsp70 intervention (28, 36, 37). We propose that the sorting signal enters the matrix, where it is specifically recognized and secured by a putative sorting signal recognition protein. The translocating preprotein then disengages from further involvement of the mt-hsp70 system for subsequent import. Evidence in favor of passage of at least N-terminal parts of mature-size cytochrome b_{2} through the matrix was recently presented; these sorting intermediates looped through the matrix while remaining in contact with components of the TIM machinery (21).

Acknowledgments—We thank Sandra Weinzierl for excellent technical assistance and Dr. Thomas Langer for helpful discussions.

REFERENCES

1. Pfanner, N., Craig, E. A., and Meijer, M. (1994) Trends Biochem. Sci. 19, 365–372
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. Bohni, P., Daum, G., and Schatz, G. (1983) J. Biol. Chem. 258, 4937–4943
5. Hawlitschek, G., Schneider, H., Schmidt, B., Tropschug, M., Hartl, F.-U., and Neupert, W. (1988) Cell 53, 795–806
6. Schleyer, M., Schmidt, B., and Neupert, W. (1982) Eur. J. Biochem. 125, 109–116
7. Maarse, A. C., Blom, J., Grivell, L. A., and Meijer, M. (1992) EMBO J. 11, 3619–3628
8. Scherer, P. E., Manning-Krieg, U., Jenö, P., Schatz, G., and Horst, M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11830–11834
9. Dekker, P. J. T., Keil, P., Rassow, J., Maarse, A. C., Pfanner, N., and Meijer, M. (1993) FEBS Lett. 330, 66–70
10. Ryan, K. R., Menold, M. M., Garrett, S., and Jensen, R. E. (1994) Mol. Biol. Cell 5, 529–538
11. Berthold, J., Bauer, M. F., Schneider, H.-C., Klaus, C., Dietmeier, K., Neupert, W., and Brunner, M. (1995) Cell 81, 1085–1093
12. Unger, C., Guider, B., Neupert, W., and Cyr, D. M. (1996) EMBO J. 15, 735–744
13. Cyr, D. M., Stuart, R. A., and Neupert, W. (1993) J. Biol. Chem. 268, 23751–23754
14. Stuart, R. A., Cyr D. M., Craig, E. A., and Neupert, W. (1994) Trends Biochem. Sci. 19, 87–92
15. Unger, C., Neupert, W., and Cyr, D. M. (1994) Science 266, 1250–1253
16. Schneider, H. C., Berthold, J., Bauer, M. F., Dietmeier, K., Guider, B., Brunner, M., and Neupert, W. (1994) Nature 371, 768–774
17. Miller, B., and Cumskey, M. G. (1993) J. Cell Biol. 121, 1021–1029
18. Schwarz, E., Seytter, T., Guider, B., and Neupert, W. (1993) EMBO J. 12, 2395–2402
19. Wachter, C., Schatz, G., and Glick, B. S. (1994) Mol. Biol. Cell 5, 465–474
20. Rojs, E. E., Stuart, R. A., and Neupert, W. (1995) EMBO J. 14, 3445–3451
21. Gruhler, A., Ono, H., Guider, B., Neupert, W., and Stuart, R. A. (1995) EMBO J. 14, 1349–1359
22. Gartner, F., Bömer, U., Guider, B., and Pfanner, N. (1995) EMBO J. 14, 6043–6057
23. Herrmann, J. M., Fülsch, H., Neupert, W., and Stuart, R. A. (1994) in Cell Biology: A Laboratory Handbook (Celis, D. E., ed) Vol. 1, pp. 538–544, Academic Press, New York
24. Gambill, B. D., Voos, W., Kang, P. J., Miao, B., Langer, T., Craig, E. A., and Pfanner, N. (1993) J. Cell Biol. 123, 109–117
25. Rassow, J., Hartl, F.-U., Guider, B., Pfanner, N., and Neupert, W. (1990) FEBS Lett. 275, 190–194
26. Pelham, H. R. B., and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247–256
27. Fülsch, H., Guider, W., Neupert, W., and Stuart, R. A. (1996) EMBO J. 15, 470–477
28. Stuart, R. A., Gruhler, A., van der Klei, I., Guider, B., Koll, H., and Neupert, W. (1994) Eur. J. Biochem. 220, 9–18
29. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
30. Laemmli, U. K. (1970) Nature 227, 680–685
31. Wagner, I., Arlt, H., van Dyck, L., Langer, T., and Neupert, W. (1994) EMBO J. 13, 5135–5145
32. Gartner, F., Voos, W., Querol, A., Miller, B. R., Craig, E. A., Cumskey, M. G., and Pfanner, N. (1995) J. Biol. Chem. 270, 3758–3766
33. Neupert, W., Hartl, F.-U., Craig, E. A., and Pfanner, N. (1990) Cell 63, 447–450
34. Glick, B. S. (1995) Cell 80, 11–14
35. Pfanner, N., and Meijer, M. (1995) Curr. Biol. 5, 132–135
36. Glick, B. S., Wachter, C., Reid, G. A., and Schatz, G. (1993) Protein Sci. 2, 1901–1971
37. Voos, W., Gambill, B. D., Guider, B., Pfanner, N., and Craig, E. A. (1993) J. Cell Biol. 123, 119–126