Efficient Folding of Firefly Luciferase after Transport into Mammalian Microsomes in the Absence of Luminal Chaperones and Folding Catalysts*

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Folding of polypeptides emerging from the protein transloca in the membrane of mammalian microsomes was analyzed after synthesis of corresponding precursor proteins in a mammalian translation system. Firefly luciferase was used as a model protein; the corresponding hybrid precursor contained the preprolactin signal peptide. The rates and efficiencies of folding of luciferase in microsomes were compared with those of folding of luciferase in the cytosol. Furthermore, folding of luciferase in microsomes was compared with folding in the cytosol. Folding in the absence of luminal molecular chaperones and folding catalysts. Folding in microsomes was less efficient compared with folding in the cytosol. Thus, firefly luciferase emerging from transloca can efficiently fold to its native conformation without chaperoning by any luminal proteins. There may be molecular chaperones present in the microsomal membrane that can efficiently substitute for the cytosolic chaperone machinery comprising Hsp40, Hsp60, and Hsp70 with respect to folding of firefly luciferase.

Little is known about protein folding and subunit assembly in the various compartments of the eukaryotic cell following de novo synthesis of polypeptides. Nevertheless, the latter is generally assumed to be assisted by molecular chaperones and folding catalysts (Ellis and van der Vies, 1991; Gething and Sambrook, 1992; Georgopoulos and Welch, 1993; Hartl et al., 1994; Helenius et al., 1994; Bergeron et al., 1994; Kunz and Hall, 1993; Schreiber, 1991). Recently, various laboratories started to address questions related to protein folding either in the mammalian cytosol, by employing rabbit reticulocyte lysate as a translation and folding system (Frydman et al., 1994; Kruse et al., 1995), or in the mammalian endoplasmic reticulum, by employing rabbit reticulocyte lysate as a translation system and dog pancreas microsomes as a folding system (Marquart et al., 1993). Specifically, it was asked what are the folding mechanisms of model proteins with light-emitting luciferases (Frydman et al., 1994; Kolb et al., 1994; Kruse et al., 1995). The first luciferase is a heterodimeric enzyme from Vibrio harveyi, and the second luciferase is a monomeric enzyme from the firefly. It was observed that (i) folding of monomeric luciferase depends on a highly organized chaperone machinery comprising Hsp40, Hsp60, and Hsp70 in the mammalian cytosol (Frydman et al., 1994) and that (ii) heterodimeric luciferase involves ATP-dependent molecular chaperones and peptidylprolyl cis-trans-isomerases in its folding and assembly in the mammalian cytosol (Kruse et al., 1995) as well as in mammalian microsomes.

To investigate whether microsomal molecular chaperones or folding catalysts can functionally substitute for the cytosolic chaperone machinery involving Hsp60, we here address questions related to folding of firefly luciferase in mammalian microsomes by employing rabbit reticulocyte lysate as a translation and folding catalysts. Firefly luciferase was specifically used here as a model protein to look at general phenomena, i.e. to exclude from the analysis molecular chaperones and folding catalysts that are specific for glycoproteins and disulfide-containing proteins (Helenius et al., 1994; Bergeron et al., 1994).

**EXPERIMENTAL PROCEDURES**

Materials—[35S]Methionine (1000 Ci/mmol) was obtained from Amersham Buchler. Cholate was purchased from Calbiochem. BspEI was from New England Biolabs Inc. All other restriction enzymes, DNA-modifying enzymes, and rabbit reticulocyte lysate were purchased from Boehringer Mannheim. SP6 polymerase, plasmid pGEMLuc, and luciferase assay reagents were from Promega. X-ray films (X-Omat AR) were from Eastman Kodak Co.

Construction of Plasmids Coding for Luciferase-related Pressecretory Proteins—Plasmids pCA37 (containing the SP6 promoter, the propeptidylprolyl cis-trans-isomerase signal peptide, and a unique BspEI site) and pCA38 (containing the SP6 promoter, the preprolactin signal peptide, and a unique BspEI site) were as described (Schlenstedt et al., 1992). For construction of plasmids coding for pluc, the Luc coding region within pGEMluc was amplified by polymerase chain reaction, and simultaneously, a piece of DNA was added on, which introduced a unique BspEI site upstream of the original initiating methionine. The polymerase chain reaction product was inserted into plasmid pSP65, resulting in plasmid pML21. A BspEI/Stul fragment, derived from pML21, was inserted into plasmids pCA37 and pCA38, which had been deaened with BspEI and PvuI, resulting in plasmids pMB10 and pMB9, respectively. All plasmid constructs were checked by DNA sequencing.

Reconstitution of Microsomal Membrane Proteins—the preparation of proteoliposomes began with ribosome-depleted microsomes, which were obtained by puromycin/high salt treatment of rough microsomes. Proteoliposomes were produced as described previously according to

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§ This abbreviation used are: pluc, artificial precursor of luciferase; Luc, firefly luciferase; gLuc, glycosylated luciferase; CS, cyclosporin A; BIP, immunoglobulin heavy chain-binding protein.
the protocol of Görlich and Rapoport (1993).

In Vitro Transcription and Translation—Transcripts of the plasmids were used to synthesize the respective proteins in a rabbit reticulocyte translation system in the presence of [35S]methionine (final concentration of 1.4 mCi/ml) or in the presence of unlabeled methionine following the supplier’s recommendations. Where indicated, dog pancreas microsomes or proteoliposomes were present during the translation. Dog pancreas microsomes and proteoliposomes were prepared as described (Schlenstedt et al., 1990). To prevent N-linked glycosylation of luciferases in microsomes, the acceptor peptide Ac-Asn-Tyr-Thr-NH2 (final concentration of 0.1 mM) was present simultaneously. The acceptor peptide was synthesized as described previously.

Analytical Methods—Reisolation of microsomes was carried out as described previously (Schlenstedt et al., 1990). Radiolabeled translation products were analyzed by electrophoresis on high Tris/SDS-polyacrylamide gels (Schlenstedt et al., 1990). The gels were treated with 1 M sodium salicylate, dried, and exposed to X-ray films. Densitometric analysis was performed with an LKB Ultrascan XL laser densitometer. Unlabeled translation products were analyzed for luciferase activity with luciferase assay reagent. Specifically, 2.5 μl of translation mixture were transferred into a luminometer vial and supplemented with 50 μl of luciferase assay reagent and 2.5 μl of 2% Triton X-100. The vial was immediately transferred into a luminometer (Berthold Model LB 9501), and bioluminescence was measured for 10 s as relative light units. Enzyme activities are given as relative light units × 10−3.

RESULTS

Folding of Firefly Luciferase in Mammalian Microsomes—

The cDNA coding for firefly luciferase was used for the construction of three artificial cDNAs. One coded for the luciferase with an additional tetrapeptide (Met-Ala-Pro-Asp) in front of the initiating methionine of firefly luciferase. The other two cDNAs coded for hybrid precursor proteins that comprised either the preprocecropin A signal peptide, the first amino acid residue of procecropin A (Ala), a dipeptide (Pro-Asp), and luciferase or the preprolactin signal peptide, the first amino acid residue of prolactin (Thr), a dipeptide (Pro-Asp), and luciferase. Thus, in both precursor constructs, the original cleavage site of either preprocecropin A or preprolactin for signal peptidase was preserved. We note that there are three potential sites for N-linked glycosylation in luciferase. Transcripts of the plasmids coding for luciferase (Luc) and the two related precursor proteins (pLuc) were used to program translation in rabbit reticulocyte lysate in the absence or presence of dog pancreas microsomes. According to the accepted rules for cotranslational amino-terminal processing in rabbit reticulocyte lysate, the signal peptide-free luciferase can be expected to contain the amino-terminal tetrapeptide Ac-Ala-Pro-Asp-Met (Met represents the initiating methionine of firefly luciferase) (Kendall et al., 1990). After processing by signal peptidase, the two luciferases can be expected to contain the amino-terminal tetrapeptides Ala-Pro-Asp-Met and Thr-Pro-Asp-Met, respectively (Schlenstedt et al., 1990).

To follow the efficiencies of synthesis and transport of the polypeptide chains, the translation reaction was carried out in the presence of [35S]methionine, and aliquots of the translation or transport reactions were taken and subjected to sequestration analysis, followed by gel electrophoresis and fluorography. The polypeptides Luc and pLuc were synthesized in the absence of microsomes, in the presence of microsomes, or in the presence of microsomes and the acceptor peptide that inhibits N-linked glycosylation (Ac-Asn-Tyr-Thr-NH2) (Figs. 1A and 2A). After synthesis of Luc under these three conditions, a single translation product was detected (Fig. 1A, lanes 1, 4, and 7). After synthesis of pLuc, comprising the preprolactin signal peptide, in the absence of microsomes, a single translation product was detected (Fig. 2A, lane 1) with the identical apparent molecular mass compared with Luc. Thus, the gels were unable to resolve pLuc and Luc (see below). After synthesis of pLuc in the presence of microsomes, two additional translation products were detected (Fig. 2A, lane 4), a major one that had a higher apparent molecular mass compared with pLuc (termed gLuc) and a minor one that had an intermediate apparent molecular mass compared with pLuc and gLuc. After synthesis of pLuc in the presence of microsomes and the inhibitory acceptor peptide, two translation products were detected (Fig. 2A, lane 7), a major product that had the identical apparent molecular mass compared with pLuc and Luc and a minor product that had an intermediate apparent molecular mass compared with pLuc and gLuc. Thus, in the presence of microsomes, pLuc was processed by signal peptidase (see below) and either glycosylated (resulting in gLuc) or not glycosylated (resulting in Luc) depending on the presence of the inhibitory acceptor peptide. We suggest that the translation product that had an intermediate apparent molecular mass compared with pLuc and gLuc corresponds to partially glycosylated Luc. The following data can be taken as evidence for the fact that trans-
of microsomes and the acceptor peptide, the active enzyme was found in the supernatant after centrifugation and was completely sensitive to protease in the absence and presence of detergent (Table 1) and by the observations that after synthesis of pluc in the presence of microsomes and the acceptor peptide, the active enzyme was found in the pellet after centrifugation and was completely resistant to protease in the absence of detergent and protease-sensitive in the presence of detergent (Table 1). Similar results were obtained with the precursor protein that contained the preprocecropin A signal peptide (data not shown). Thus, folding of luciferase to the native state occurred after synthesis of pluc in reticulocyte lysate and concomitant with transport into dog pancreas microsomes. On the basis of parallel experiments that were carried out in the presence of [35S]methionine, apparent specific activities were calculated for luciferase after synthesis of Luc and pluc in the presence of microsomes and the acceptor peptide (i.e. by dividing activity by the relevant amount of protein). The apparent specific activity of luciferase after folding in the cytosol (14.5) was found to be twice as high compared with the apparent specific activity of luciferase after folding in microsomes (7.2).

Folding of Luciferase Is Efficient in Proteoliposomes—To determine the possible involvement of molecular chaperones and folding catalysts in luciferase folding in mammalian microsomes, the kinetics of folding were studied after transport into proteoliposomes. These proteoliposomes were previously observed to be depleted with respect to luminal proteins, i.e. ATP-dependent molecular chaperones and folding catalysts (Görlich and Rapoport, 1993). The precursor pluc was synthesized in the presence of the acceptor peptide and either microsomes or proteoliposomes. To follow the efficiencies of transport of the precursor polypeptide, the translation reaction was carried out in the presence of [35S]methionine, and aliquots of the translation reactions were taken and subjected to sequestration analysis, followed by gel electrophoresis and fluorography (Fig. 3A). The data from the sequestration analysis can be taken as an indication of the fact that transport of the precursor protein into the proteoliposomes had occurred. To follow the kinetics of folding, the translation was carried out in the presence of a labeled amino acid, and aliquots were taken and analyzed in the presence of the required substrates of the luciferase reaction by luminometry. To determine the location of the active luciferases, the translation was carried out in the presence of microsomes and the acceptor peptide. Transcripts of plasmid pML21 were used to synthesize Luc in rabbit reticulocyte lysate at 30°C in the presence of microsomes and the acceptor peptide. Transcripts of plasmid pMB9 were used to synthesize pluc in rabbit reticulocyte lysate at 30°C in the presence of microsomes and the acceptor peptide. Transcripts of plasmid pMB9 were used to synthesize pluc in rabbit reticulocyte lysate at 30°C in the presence of microsomes and the acceptor peptide. Transcripts of plasmid pMB9 were used to synthesize pluc in rabbit reticulocyte lysate at 30°C in the presence of microsomes and the acceptor peptide.

### Table 1

| Condition               | Specific Activity (in luciferase units) |
|-------------------------|----------------------------------------|
| Supernatant             | 42.838                                 |
| Pellet                  | 42.293                                 |
| Protease + Triton X-100 | 1.900                                  |
| Total                   | 16.648                                 |
| Total                   | 42.838                                 |
| Supernatant             | 24.515                                 |
| Pellet                  | 6.646                                  |
| Protease + Triton X-100 | 9.609                                  |
| Total                   | 20.572                                 |

**Fig. 2.** Folding of Luc after synthesis of pluc in the presence of dog pancreas microsomes. Transcripts of plasmid pMB9 were used to synthesize pluc in rabbit reticulocyte lysate under the conditions described in the legend to Fig. 1. Protein synthesis, transport, and folding were evaluated and are shown as described in the legend to Fig. 1.

In the first set of folding experiments, the kinetics of synthesis of Luc and synthesis and transport of pluc were compared with the kinetics of luciferase folding. We observed that luciferase folding was as fast as protein synthesis or synthesis and transport (data not shown). In the second set of folding experiments, Luc and pluc were synthesized in the absence of microsomes, in the presence of microsomes, or in the presence of microsomes and the acceptor peptide. According to the folding kinetics of Luc, efficient folding occurred in the absence as well as presence of microsomes and irrespective of whether the acceptor peptide was present or not (Fig. 1B). According to the folding kinetics of pluc, efficient folding occurred only in the presence of microsomes and the acceptor peptide (Fig. 2B). Apparently, the presence of the signal peptide as well as the glycosylation of the mature protein interfered with the folding reaction. This view was supported by fractionation and sequestration analysis, i.e. by the observations that after synthesis of Luc in the presence of microsomes and the acceptor peptide, the active enzyme was found in the supernatant after centrifugation and was completely sensitive to protease in the absence and presence of detergent (Table 1) and by the observations that after synthesis of pluc in the presence of microsomes and the acceptor peptide, the active enzyme was found in the pellet after centrifugation and was completely resistant to protease in the absence of detergent and protease-sensitive in the presence of detergent (Table 1). Similar results were obtained with the precursor protein that contained the preprocecropin A signal peptide (data not shown). Thus, folding of luciferase to the native state occurred after synthesis of pluc in reticulocyte lysate and concomitant with transport into dog pancreas microsomes. On the basis of parallel experiments that were carried out in the presence of [35S]methionine, apparent specific activities were calculated for luciferase after synthesis of Luc and pluc in the presence of microsomes and the acceptor peptide (i.e. by dividing activity by the relevant amount of protein). The apparent specific activity of luciferase after folding in the cytosol (14.5) was found to be twice as high compared with the apparent specific activity of luciferase after folding in microsomes (7.2).

Folding of Luciferase Is Efficient in Proteoliposomes—To determine the possible involvement of molecular chaperones and folding catalysts in luciferase folding in mammalian microsomes, the kinetics of folding were studied after transport into proteoliposomes. These proteoliposomes were previously observed to be depleted with respect to luminal proteins, i.e. ATP-dependent molecular chaperones and folding catalysts (Görlich and Rapoport, 1993). The precursor pluc was synthesized in the presence of the acceptor peptide and either microsomes or proteoliposomes. To follow the efficiencies of transport of the precursor polypeptide, the translation reaction was carried out in the presence of [35S]methionine, and aliquots of the translation reactions were taken and subjected to sequestration analysis, followed by gel electrophoresis and fluorography (Fig. 3A). The data from the sequestration analysis can be taken as an indication of the fact that transport of the precursor protein into the proteoliposomes had occurred. To follow the kinetics of folding, the translation was carried out in the presence of a labeled amino acid, and aliquots were taken and analyzed in the presence of the required substrates of the luciferase reaction by luminometry. To determine the location of the active luciferases, the translation was carried out in the presence of microsomes and the acceptor peptide.
absence of a labeled amino acid, and aliquots were taken and subjected to sequestration analysis, followed by luminometry (Table II). The data from the sequestration analysis can be taken as an indication of the fact that folding in the lumen of proteoliposomes was analyzed under these conditions. To be able to directly compare the efficiencies of luciferase folding in microsomes and proteoliposomes, the folding kinetics were corrected for identical luciferase contents of microsomes and proteoliposomes (Fig. 3B). Thus, folding of luciferase in proteoliposomes was approximately twice as efficient compared with folding in microsomes.

Folding of Luciferase in Microsomes Is Not Inhibited by Pretreatment of Microsomes with Either 8-Azido-ATP plus UV Light or CsA plus FK506—To corroborate the lack of a role of luminal molecular chaperones and peptidylprolyl cis/trans-isomerases in luciferase folding in the microsomes, the folding kinetics were corrected for identical luciferase contents of microsomes and proteoliposomes (Fig. 3B). Thus, folding of luciferase in proteoliposomes was approximately twice as efficient compared with folding in microsomes.

Folding of Luciferase in Microsomes Is Not Inhibited by Pretreatment of Microsomes with Either 8-Azido-ATP plus UV Light or CsA plus FK506—To corroborate the lack of a role of luminal molecular chaperones and peptidylprolyl cis/trans-isomerases in luciferase folding in the microsomes, the folding kinetics of luciferase were studied after pretreatment of microsomes with inhibitors of either ATP-dependent molecular chaperones (8-azido-ATP plus UV light) (Klappa et al., 1991; Clairmont et al., 1992) or peptidylprolyl cis/trans-isomerases (CsA and FK506) (Kunz and Hall, 1993; Schreiber, 1991). The pretreatment with 8-azido-ATP plus UV light interfered (−60% inhibition) and the pretreatment with CsA and FK506 did not interfere with subsequent protein transport (data not shown). In the first experiment, the precursor pLuc was synthesized in the presence of microsomes and the acceptor peptide and in the additional presence of either Me2SO or CsA plus FK506 (Fig. 4A). The enzyme activities were measured and corrected for identical luciferase contents in the two types of microsomes (as described above). Folding of Luc was not sensitive to the two drugs. We conclude from this set of data that peptidylprolyl cis/trans-isomerases are not involved in folding of Luc. Furthermore, we conclude that proline isomerization does not play

FIG. 3. Folding of Luc in proteoliposomes. Transcripts of plasmid pMB9 were used to synthesize pLuc in rabbit reticulocyte lysate at 30 °C in the presence of either rough microsomes (A, RM; and B, open circles) or proteoliposomes (A, PL; and B, closed circles) plus the acceptor peptide (acetyl-Asn-Tyr-Thr-NH2, (NYT)). In A and B, protein synthesis, transport, and folding were evaluated as described in the legend to Fig. 1. However, the activity data were corrected for identical protein contents (shown in lanes 2 and 5 of A).

| Table II | Localization of enzymatically active luciferase after synthesis of pLuc in the presence of microsomes or proteoliposomes |
|----------------------------------|----------------------------------|
| Activity after synthesis of pLuc in the presence of | |
| Microsomes | Proteoliposomes |
| Total | 7.402 | 5.439 |
| Protease | 7.785 | 4.568 |
| Protease + Triton X-100 | 1.363 | 0.377 |

FIG. 4. Folding of Luc in microsomes is not sensitive to CsA plus FK506 and to photoaffinity labeling with 8-azido-ATP. A, microsomes were incubated for 20 min at 0 °C either with Me2SO (final concentration of 1%) (open diamonds) or with CsA plus FK506 (final concentration of 100 μg/ml) (closed diamonds) that were solubilized in Me2SO. B, microsomes were either supplemented with 8-azido-ATP (final concentration of 5 mM) (open squares) or photoaffinity-labeled with 8-azido-ATP (closed squares) for 1 min as described previously (Klappa et al., 1991). In A and B, transcripts of plasmid pMB9 were used to synthesize pLuc in rabbit reticulocyte lysate at 30 °C in the presence of microsomes and the acceptor peptide. At the indicated times of translation, aliquots (2.5 μl) were withdrawn and analyzed for luciferase activity. Translation was also carried out in the presence of [35S]methionine. After 60 min, the translation reaction was divided into three aliquots. One aliquot was incubated further in the absence of protease, a second one in the presence of protease, and a third one in the presence of protease plus detergent. After inhibition of the protease, all samples were analyzed by gel electrophoresis and fluorography. The activity data were corrected for identical protein contents.
a role in folding of Luc. This finding is consistent with the observation that cis/trans-isomerization of proline-containing peptide bonds is not rate-limiting during luciferase refolding (Kolb et al., 1994). In the second experiment, the precursor pLuc was synthesized in the presence of the acceptor peptide and in the additional presence of microsomes pretreated with 8-azido-ATP in the absence or presence of UV light (Fig. 4B). The enzyme activities were measured and corrected for identical luciferase activities in the two types of microsomes (as described above). Folding of Luc was approximately twice as efficient in microsomes that had been photoaffinity-labeled with 8-azido-ATP compared with untreated microsomes.

**DISCUSSION**

Folding of firefly luciferase in the mammalian cytosol had previously been shown to involve a highly organized chaperone machinery comprising Hsp40, Hsp60, and Hsp70 (Frydman et al., 1994). Up to now, there has been no indication of a member of the Hsp60 protein family present in the endoplasmic reticulum. To investigate which microsomal molecular chaperones or folding catalysts can functionally substitute for the cytosolic chaperone machinery, folding of firefly luciferase, emerging from the protein translocase in the membrane of mammalian microsomes, was analyzed after synthesis of the corresponding hybrid precursor protein in a mammalian translation system. Firefly luciferase was specifically used here as a model protein in order to exclude from the analysis molecular chaperones and folding catalysts that are specific for glycoproteins and disulfide-containing proteins. The rates and efficiencies of folding of luciferase in the cytosol were compared with those of folding of luciferase in microsomes and in microsomes that had been pretreated with inhibitors of either ATP-dependent molecular chaperones (such as BiP) or peptidylprolyl cis/trans-isomerases (cyclophilins and FK506-binding proteins). Inhibition of such molecular chaperones had previously been shown to affect folding of heterodimeric luciferase in mammalian cytosol and microsomes (Kruse et al., 1995). Furthermore, folding of luciferase in microsomes was compared with folding in proteoliposomes, i.e. in the absence of luminal molecular chaperones (such as BIP) and folding catalysts (such as cyclophilins and FK506-binding proteins). The rate and efficiency of folding of heterodimeric luciferase in the absence of luminal chaperones were previously found to be dramatically reduced compared with those of folding in the presence of luminal proteins. Furthermore, it was observed that the residual folding of heterodimeric luciferase in proteoliposomes was not sensitive to inhibitors of BiP and peptidylprolyl cis/trans-isomerases.

Folding of firefly luciferase in microsomes was as fast as but less efficient than folding in the cytosol (Frydman et al., 1994; Kolb et al., 1994). Folding of firefly luciferase in the absence of luminal proteins and after inhibition of ATP-dependent molecular chaperones was more efficient compared with folding in the presence of luminal molecular chaperones and was indistinguishable from folding in the cytosol. Therefore, we suggest that a luminal protein with an affinity for 8-azido-ATP, most likely BiP (Clairmont et al., 1992), interfered with luciferase folding in microsomes. Thus, firefly luciferase emerging from translocase can efficiently fold to its native conformation without any help from soluble molecular chaperones. Therefore, we conclude that there are molecular chaperones present in the microsomal membrane that can efficiently substitute for the cytosolic chaperone machinery with respect to folding of firefly luciferase. However, an alternative interpretation has to be considered. The possibility cannot be dismissed that firefly luciferase can spontaneously fold to its native state with high efficiency under conditions of its de novo synthesis. We favor the first possibility since spontaneous folding of luciferase after denaturation and subsequent renaturation of the purified enzyme was found to be slow (half-time of 14 min to hours, depending on the conditions) (Kolb et al., 1994).

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**REFERENCES**

Bergeron, J. M., Brenner, M. B., Thomas, D. Y., and Williams, D. B. (1994) Trends Biochem. Sci. 19, 124–128
Clairmont, C., De Maio, A., and Hirschberg, C. B. (1992) J. Biol. Chem. 267, 3983–3990
Ellis, R. J., and van der Vies, S. M. (1991) Annu. Rev. Biochem. 60, 321–347
Frydman, J., Nimmesgern, E., Ohtsuka, K., and Hartl, F. U. (1994) Nature 370, 111–117
Gething, M.-J., and Sambrook, J. (1992) Trends Biochem. Sci. 17, 33–45
Görlich, D., and Rapoport, T. A. (1993) Cell 75, 615–630
Hartl, F.-U., Hlodan, R., and Langer, T. (1994) Trends Biochem. Sci. 19, 20–25
Helenius, A., Marquart, T., and Braakman, I. (1994) Trends Biochem. Sci. 19, 227–231
Kendall, R. L., Yamada, R., and Bradshaw, R. A. (1990) Methods Enzymol. 185, 398–407
Klappa, P., Mayinger, P., Pippkon, R., Zimmermann, M., and Zimmermann, R. (1993) EMBO J. 12, 2799–2803
Kolb, V. A., Makeyev, E. V., and Spirin, A. S. (1994) EMBO J. 13, 3631–3637
Krusz, M., Brunke, M., Escher, A., Szalay, A. A., Tropschug, M., and Zimmermann, R. (1995) J. Biol. Chem. 270, 2588–2594
Kurtz, J., and Hall, M. N. (1993) Trends Biochem. Sci. 18, 334–338
Marquart, T., Hebert, D. N., and Helenius, A. (1993) J. Biol. Chem. 268, 19618–19625
Schlenstedt, G., Gudmundsson, G. H., Boman, H. G., and Zimmermann, R. (1990) J. Biol. Chem. 265, 13960–13968
Schlenstedt, G., Gudmundsson, G. H., Boman, H. G., and Zimmermann, R. (1992) J. Biol. Chem. 267, 24328–24332
Schreiber, S. L. (1991) Science 251, 283–287

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3 R. Herbst, U. Schäfer, and R. Seckler, manuscript in preparation.