Membrane Topology and Retention of Microsomal Aldehyde Dehydrogenase in the Endoplasmic Reticulum*

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Microsomal aldehyde dehydrogenase (msALDH) is anchored to the endoplasmic reticulum (ER) membrane by the hydrophobic domain at its carboxyl terminus, and most of the molecule is exposed to the cytoplasm (Masaki, R., Yamamoto, A., and Tashiro, Y. (1994) J. Cell Biol. 126, 1407–1420). To determine the membrane topology and the intracellular localization of msALDH, the amino-terminal region of bovine opsin containing N-glycosylation sites was fused to the carboxyl terminus of msALDH, and three chimeric proteins with extensions of different sizes were expressed in COS cells. Indirect immunofluorescence microscopy showed the ER localization of all of the chimeric proteins similar to wild-type msALDH. Immunoblotting revealed that the two chimeric proteins containing longer extensions, those with the N-glycosylation site at distances of 13 and 21 amino acids from the membrane anchor, respectively, were N-glycosylated. These results indicate that the membrane binding domain of msALDH spans the bilayer of the ER. The carbohydrate chain of the chimeras was sensitive to endoglycosidase H but resistant to endoglycosidase D. Upon treatment of transfected COS cells with brefeldin A, the carbohydrate chain was processed to an endoglycosidase H-resistant form, presumably by cis/medial Golgi-specific enzymes redistributed in the ER. These biochemical results in addition to immunofluorescence microscopic observations suggest that msALDH is retained in the ER by blocking of the exit from the ER.

EXPERIMENTAL PROCEDURES

Materials—Fetal bovine serum and Dulbecco’s minimal essential medium were purchased from Flitton Co. (Brooklyn, Australia) and Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Brefeldin A and antibiotics were obtained from Epicenter Technologies (Madison, WI) and Life Technologies, Inc., respectively. [35S]Metionine-cysteine was from DuPont NEN.

Goat anti-rabbit IgG conjugated with peroxidase was purchased from Tago, Inc. (Burlingame, CA). Goat anti-rabbit IgG conjugated with rhodamine and anti-mouse IgG conjugated with fluorescein were obtained from Protos Immunoresearch (San Francisco) and American Qualex Antibodies & Immunodechemicals Co. (La Mirada, CA), respectively. Mouse monoclonal antibodies against human protein disulfide isomerase were from Fuji Yakuhin Kogyo Co., Ltd. (Toyama, Japan). Rabbit antibodies against rat liver msALDH were obtained and characterized as described (7).
Protein A-Sepharose 4B was from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden). Endoglycosidase H (endo H), endoglycosidase D (endo D), and N-glycosidase F were purchased from Boehringer Mannheim. Restriction enzymes and DNA-modifying enzymes were obtained from Nippon Gene (Toyama, Japan) and Takara Co., Ltd. (Kyoto, Japan). DNA sequencing kits were from U. S. Biochemical Corp. Oligonucleotide synthesis was carried out with an Applied Biosystems model 381A DNA synthesizer. All other chemicals were of the highest purity commercially available.

The cDNA encoding bovine opsin in the pSP vector and a 50% suspension of Staphylococcus aureus aureus were kindly provided by Dr. Takashi Morimoto (New York University) and Dr. Shigeru Taketani (Kagoshima University, Osaka, Japan), respectively.

Plasmid Constructions—All constructions were verified by the diode chain termination method (15) and restriction enzyme digestion. The full-length cDNA for rat msALDH (7) was inserted into the HindIII-EcoRV sites of the mammalian expression vector, pMIW (16), to construct pMIWALDH. Chimeric cDNAs between msALDH and bovine opsin were constructed by the gapped duplex method of oligonucleotide-directed mutagenesis (17), followed by either annealing of oligonucleotides or the polymerase chain reaction. Synthetic oligonucleotide 1 (5'-TCAAGATCACTCAGGCTGACTGAGCTCT-ACTGGA-3'), with the mutated nucleotides underlined, was used for the generation of XbaI and Accl sites at the carboxyl terminus of msALDH. The mutated cDNA was inserted into pMIW digested with HindIII and EcoRV to construct pMIWALDH.XA, which contains the msALDH with an additional Ser-Arg-Val-Asp (SRVD) sequence (amino acids 485-488) at its carboxyl terminus.

For construction of pMIWALDH/OP1, oligonucleotides 2 (5'-CTGCTATGCTACGGTGCTGACTGAGCTCTA-3') and 3 (5'-TTTATCGATCAGGTCCCT-3') were annealed, digested with XbaI and Accl, and then ligated into the XbaI-Accl site of pMIWALDH.XA. Similarly, oligonucleotides 4 (5'-CGTCTAGATCAACCAAAGGCGCTGACTGAGCTCTA-3') and 5 (5'-TCTTATCGATCAGGTCCCTCTTGTGACTACAG-3') were used to create pMIWALDH/OP1.TK. pMIWALDH/OP2 was produced from oligonucleotides 6 (5'-CGTCTATGCTATGTGCTGGCTGACTGAGCTCTA-3') and 7 (5'-GCGATCCAAGAAGGTCGACTGATGAA-3') for the generation of pMIWALDH/OP2TK. pMIWALDH/OP3 was produced from oligonucleotides 8 (5'-AAAAGATCACTGAGCTCCTTGTGGTAGTGACAG-3') and 9 (5'-TCTTATGCTGAGCTCCTTGTGGTAGTGACAG-3') to create pMIWALDH/OP3.TK, pMIWALDH/OP3TK was produced from oligonucleotides 10 (5'-CGTCTAGATCAACCAAAGGCGCTGACTGAGCTCTA-3') and 11 (5'-TCTTATGCTGAGCTCCTTGTGGTAGTGACAG-3') for the generation of pMIWALDH/OP3TK. Polymerase chain reactions involving the following primers and templates were performed to amplify DNA fragments termed OPZTK, OP3, and OPZTK: OPZTK, oligonucleotides 8 (5'-AAAAGATCACTGAGCTCCTTGTGGTAGTGACAG-3') and 9 (5'-TCTTATGCTGAGCTCCTTGTGGTAGTGACAG-3') with the mutated nucleotides underlined, and pMIWALDH/OP2 as a template; OP3, oligonucleotides 10 (5'-CGTCTAGATCAACCAAAGGCGCTGACTGAGCTCTA-3') and 11 (5'-TCTTATGCTGAGCTCCTTGTGGTAGTGACAG-3') with the mutated nucleotides underlined, and pMIWALDH/OP3 as a template. The XbaI-Accl fragments of polymerase chain reaction products were ligated into pMIWALDH.XA digested with XbaI and Accl. The resultant plasmids were designated as pMIWALDH/OPZTK, pMIWALDH/OP3, and pMIWALDH/OPZTK, respectively.

Expression in COS Cells—The transfection of COS cells, subcellular fractionation, membrane extractions, and indirect immunofluorescence microscopy were performed as described previously (9). Samples were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (18), followed by immunoblotting as described (9).

Biologic labeling of COS Cells and Immunoprecipitation—Forty-four hours after transfection, the cells were bio-chemically activated at 37°C with or without brefeldin A (10 μg/ml) in Dulbecco's minimal essential medium devoid of methionine and fetal bovine serum and then pulse labeled for 30 min in the same medium containing 200 μCi/ml [35S]methionine. After labeling, the cells were chased with or without brefeldin A in complete medium containing 10% fetal bovine serum for 3 h, washed three times with cold phosphate-buffered saline, and then lysed in 10 ml Tris-HCl, pH 7.5, 0.15 M NaCl, 1% Nonidet P-40, 5 mM EDTA, 1% Trasylol, 1 μg phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol for 15 min on ice. The lysates were centrifuged for 30 min at 10,000 × g; the resulting supernatants were incubated with the S. aureus suspension for 30 min on a rotating device at 4°C. Then ALDH/OP3 was immunoprecipitated from the clarified medium by the addition of rabbit anti-msALDH antibodies followed by incubation with protein A-Sepharose 4B for 2 h. Immunoprecipitates were washed four times with RIPA buffer (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.3% CHAPS, and 2 mM EDTA). The proteins were eluted by heating at 100°C for 2 min in SDS and 1% -mercaptoethanol.

Endoglycosidase and Glycosidase Treatments—For endo H treatment, the eluates were adjusted to 50 mM sodium citrate, pH 5.5, 1% N-glycosidase F, and 0.1% w/v thioflavin-C. After incubation at 37°C for 3 h, the samples were cooled on ice and then resolved by 5% SDS-PAGE and fluorography.
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expressed proteins by immunoblotting. Forty-four hours after transfection, the cells were harvested and subjected to subcellular fractionation. In this case, the postnuclear supernatant was centrifuged at 88,000 × g for 80 min to separate the membrane fraction from the cytosol fraction. As expected, the three chimeric proteins as well as wild-type msALDH were recovered exclusively in the membrane fraction (Fig. 3A). In addition, these mutants in the membrane fraction were resistant to alkali extraction (Fig. 3B), showing that the three chimeras as well as msALDH are integral membrane proteins. These results, in agreement with indirect immunofluorescence microscopic data, also indicate that the carboxyl-terminal extensions do not interfere with integration of the chimeric proteins into the ER membrane. As shown in Fig. 3A (lanes 1 and 3), ALDH/OP1 exhibited a mobility almost identical to that of msALDH (54 kDa). Two bands were observed for ALDH/OP2 (lane 5): one with a mobility corresponding to that of the protein moiety (55 kDa) of the chimera and a slower migrating form with a mobility shift of about 3 kDa. The mobility of ALDH/OP3 was also shifted about 3 kDa compared with that of its protein moiety (56 kDa) (lane 7). These results suggest that Asn-493 of ALDH/OP2 and Asn-501 of ALDH/OP3 are glycosylated, whereas Asn-488 of ALDH/OP1 and Asn-488 of ALDH/OP3 are not.

To confirm the N-glycosylation of ALDH/OP2 and ALDH/OP3, we constructed an additional series of ALDH/OP chimeras (TK mutants, ALDH/OP1TK-3TK) (Fig. 4A), in which each threonine residue (Thr-490 of ALDH/OP1, Thr-495 of ALDH/OP2, or Thr-503 of ALDH/OP3) in the N-glycosylation signal (consensus sequence Asn-X-Thr) was replaced by the underlined lysine residue. These mutants were constructed as described under “Experimental Procedures.” The single amino acid code is used, and the amino acid numbers are shown on top of each sequence. Panel B, COS cells were transfected with DNA encoding the wild-type or the mutated form of msALDH and harvested 44 h after transfection. Each homogenate was resolved by SDS-PAGE and immunoblotted using anti-msALDH antibodies. Lane 1, msALDH; lane 2, ALDH/OP1; lane 3, ALDH/OP1TK; lane 4, ALDH/OP2; lane 5, ALDH/OP2TK; lane 6, ALDH/OP3; lane 7, ALDH/OP3TK.

Fig. 2. Immunofluorescence localization of msALDH and ALDH/OP3. COS cells were transfected with msALDH (panels A and B) or ALDH/OP3 (panels C and D) in the pMIW expression vector, fixed 44 h after transfection, and then permeabilized. The expressed proteins were then detected by incubation with anti-msALDH antibodies, followed by with rhodamine-conjugated IgG (panels A and C). Endogenous protein disulfide isomerase was stained with the corresponding monoclonal antibodies followed by with fluorescein-conjugated IgG (panels B and D).

Fig. 3. Immunoblot analysis of msALDH and ALDH/OP1–3. COS cells transfected with cDNA encoding msALDH or ALDH/OP1–3 in the pMIW expression vector were harvested 44 h after transfection. Panel A, the membrane (pellet) and cytosol (supernatant) fractions were prepared by centrifugation of the postnuclear fraction at 88,000 × g for 80 min. Panel B, the membrane fractions were treated with 100 mM Na2CO3 at 0 °C for 30 min and then centrifuged at 88,000 × g for 80 min to separate the pellet from the supernatant. Each fraction was assayed by immunoblotting using anti-msALDH antibodies. Lane 1, msALDH (pellet); lane 2, msALDH (supernatant); lane 3, ALDH/OP1 (pellet); lane 4, ALDH/OP1 (supernatant); lane 5, ALDH/OP2 (pellet); lane 6, ALDH/OP2 (supernatant); lane 7, ALDH/OP3 (pellet); lane 8, ALDH/OP3 (supernatant).

Fig. 4. Immunoblot analysis of the wild-type and mutated forms of msALDH. Panel A, the carboxyl-terminal sequences of TK mutants, in which each threonine residue in the N-glycosylation signal was replaced by the underlined lysine residue. These mutants were constructed as described under “Experimental Procedures.” The single amino acid code is used, and the amino acid numbers are shown on top of each sequence. Panel B, COS cells were transfected with DNA encoding the wild-type or the mutated form of msALDH and harvested 44 h after transfection. Each homogenate was resolved by SDS-PAGE and immunoblotted using anti-msALDH antibodies. Lane 1, msALDH; lane 2, ALDH/OP1; lane 3, ALDH/OP1TK; lane 4, ALDH/OP2; lane 5, ALDH/OP2TK; lane 6, ALDH/OP3; lane 7, ALDH/OP3TK.
by Since glycoproteins become sensitive to endo D after processing in the Golgi compartment and then is recycled back to the ER, immunoprecipitated proteins from COS cells were pulse labeled for 30 min and subsequently chased for 3 h in complete medium. After immunoprecipitation, the proteins were analyzed directly by SDS-PAGE (lane 1) or after incubation overnight with endo H (lane 2) or endo D (lane 3). When the effect of brefeldin A was investigated, cells treated with brefeldin A (10 μg/ml) were pulse labeled for 30 min and subsequently chased for 3 h in complete medium containing brefeldin A. After immunoprecipitation, the proteins were analyzed either by SDS-PAGE (lane 4) or after incubation overnight with endo H (lane 5) or N-glycosidase F (lane 6).

![Figure 5](image_url)

**Fig. 5. Processing of newly synthesized ALDH/OP3 in control or brefeldin A (BFA)-treated COS cells.** COS cells were transfected with ALDH/OP3 in the pM1W expression vector. Forty-four hours later, the cells were pulse labeled for 30 min and subsequently chased for 3 h in complete medium. After immunoprecipitation, the proteins were either analyzed directly by SDS-PAGE (lane 1) or after incubation overnight with endo H (lane 2) or endo D (lane 3). When the effect of brefeldin A was investigated, cells treated with brefeldin A (10 μg/ml) were pulse labeled for 30 min and subsequently chased for 3 h in complete medium containing brefeldin A. After immunoprecipitation, the proteins were either analyzed directly by SDS-PAGE (lane 4) or after incubation overnight with endo H (lane 5) or N-glycosidase F (lane 6).

Carboxy-terminal sequence of six tail-anchored proteins, including the four proteins described above. Since the hydrophobic domains of these proteins differ in length (16–23 amino acids) and the composition of the carboxy-terminal membrane binding domain of msALDH spans the phospholipid bilayer and that the carboxy terminus is located in the luminal side of the ER. We have also demonstrated that the efficiency of glycosylation depends on the distance between the N-glycosylation site and the membrane anchor. Asn-488, located at a distance of 8 amino acids from the membrane anchor, was not glycosylated, whereas asparagine located at a distance of 13 or 21 was glycosylated. These data suggest that Asn-488 is too close to the membrane to be glycosylated by oligosaccharyltransferase.

Processing of the Newly Synthesized ALDH/OP3 Chimera—We analyzed the carbohydrate structure of ALDH/OP3 to examine its intracellular localization. Forty-four hours after transfection, COS cells were pulse labeled for 30 min with [35S]methionine-cysteine and chased for 3 h, and then the radiolabeled chimera was immunoprecipitated with anti-msALDH antibodies. As shown in Fig. 5 (lane 1), two major products were immunoprecipitated: one with the molecular mass of the unglycosylated ALDH/OP3 (56 kDa), and a slower migrating one with the size expected for its glycosylated form.

Upon endo H treatment of the immunoprecipitated proteins, the upper species was shifted to the position of the lower one (lane 2), demonstrating that the upper product is indeed glycosylated and that the carbohydrate structure of the chimera is of a high mannose type. These results suggest that ALDH/OP3 does not reach the medial Golgi compartment, where the modification of glycoproteins to endo H-resistant forms occurs (21).

To rule out the possibility that the chimera reaches the cis Golgi compartment and then is recycled back to the ER, immunoprecipitated proteins were digested with endo D. As shown in Fig. 5 (lane 3), the glycosylated ALDH/OP3 was resistant to endo D. Since glycoproteins become sensitive to endo D after processing by α-mannosidase 1A, which is supposed to be located in the cis Golgi (22), these results suggest that the chimera does not reach the cis Golgi compartment.

To confirm further that ALDH/OP3 is retained in the ER, metabolic labeling was performed in the presence of brefeldin A, an inhibitor of ER-Golgi transport (23). It is well known that glycoproteins retained in the ER are processed rapidly by redistribution cis/medial Golgi enzymes in brefeldin A-treated cells (24). Therefore, this reagent is a good indicator for proving the ER retention of ALDH/OP3. Upon treatment of transfected COS cells with brefeldin A, the chimera took on a form with a heterogeneous mobility insensitive to endo H (Fig. 5, lanes 4 and 5). The smear disappeared on digestion of the immunoprecipitates with N-glycosidase F (lane 6), an enzyme known to remove all N-linked carbohydrate chains, indicating that ALDH/OP3 was indeed processed by cis/medial Golgi enzymes redistributed to the ER as a result of the brefeldin A treatment. These biochemical results suggest that ALDH/OP3 is not transported to the cis Golgi compartment but is retained in the ER through a mechanism different from the retrieval or recycling model (10–12).

**DISCUSSION**

Native or artificially introduced N-glycosylation has been used to determine the membrane topology of proteins, especially those with multiple membrane-spanning domains (25). By taking advantage of this strategy, we have shown here that the carboxy-terminal membrane binding domain of msALDH spans the phospholipid bilayer and that the carboxy terminus is located in the luminal side of the ER. We have also demonstrated that the efficiency of glycosylation depends on the distance between the N-glycosylation site and the membrane anchor. Asn-488, located at a distance of 8 amino acids from the membrane anchor, was not glycosylated, whereas asparagine located at a distance of 13 or 21 was glycosylated. These data suggest that asn-488 is too close to the membrane to be glycosylated by oligosaccharyltransferase, which is composed of ribophorins I and II and a third 48-kDa protein (26). This result is in agreement with that obtained by Nilsson and von Heijne (27), who showed that the N-glycosylation site should be at least 12–14 amino acids away from the membrane anchor by in vitro transcription/translation of model proteins in the presence of rough microsomes. Since msALDH is post-translationally targeted by the ER-targeting sequences located on either side of the carboxy-terminal membrane binding domain and inserted into the ER (9), it is noteworthy that N-glycosylation takes place not only cotranslationally during the translocation of nascent polypeptides but also post-translationally after insertion of the carboxy-terminal hydrophobic domain of ALDH/OP3 chimeras into the ER membrane.

Recent studies have shown that the carboxy termini of two tail-anchored proteins, microsomal cytochrome b_{5} (28) and synaptobrevin (29), exhibit a luminal orientation. After insertion into the ER membrane in a signal recognition particle- and Sec61-independent fashion, synaptobrevin is transported to synaptic-like vesicles through the Golgi apparatus in PC12 cells (29). Additionally, we have shown that HPC-1/syntaxin 1A (30) is transported to the plasma membrane after post-translational insertion into the ER through the carboxy-terminal hydrophobic domain and that this protein has a similar membrane topology. Although the transmembrane topology of only the four tail-anchored proteins has been clarified at present, it appears that most tail-anchored proteins have a similar topology. Fig. 6 shows the alignment of the carboxy-terminal sequences of six tail-anchored proteins, including the four proteins described above. Since the hydrophobic domains of these proteins differ in length (16–23 amino acids) and the composition of the carboxy-terminal sequence of six tail-anchored proteins, including the four proteins described above. Since the hydrophobic domains of these proteins differ in length (16–23 amino acids) and the composition...
tion of hydrophobic amino acids, it is less likely that these domains are important determinants for the transmembrane topology. Probably, they function as targeting or retention signals to localize to their final destinations after insertion into the ER membrane. Similarly, the carboxyl termini of these proteins exhibit no common feature. Cytochrome b$_5$ (31), msALDH (7), and SS01 (32) have charged residues, whereas the other three proteins do not. On the contrary, positively charged residues are commonly located on the amino-terminal side of the hydrophobic domain with the exception in cytochrome b$_5$, suggesting the important role of positively charged residues in the translocation of the hydrophobic domains across the ER membrane. This agrees with the positive inside rule (33), which states that positively charged residues are preferentially found on the cytoplasmic sides of membrane proteins. However, the detail mechanism for the targeting of tail-anchored proteins to the ER and for integration of the carboxyl-terminal hydrophobic domains into the ER membrane remains to be resolved.

It is widely accepted that there are two mechanisms for the localization of ER resident proteins; one is the retrieval mechanism from the intermediate compartment, and the other is the retention mechanism, that is, the blocking of exit from the ER. The carboxyl-terminal KDEL sequence of luminal ER proteins functions as a retrieval signal from the intermediate compartment to the ER (10, 11). In addition, the d-lysine motif (KKXX or KXKXX) on the cytoplasmic side of some ER membrane proteins also serves as a retrieval signal (32). On the other hand, the ER retention signal of P450 has been defined. P450 is inserted into the ER membrane by its amino-terminal signal anchor sequence (2–4), which also functions as an ER retention signal (14, 34). Additionally, recent studies have shown that the cytoplasmic and amino-terminal transmembrane domains of P450 contain independent redundant signals for retention in the ER (35) and that P450 is retained in the ER without recycling through the intermediate or the cis Golgi compartment (5, 13, 14). In this study, N-glycosylation of ALDH/OP chimeras provided a powerful tool for determining the mechanism for their ER localization. To the best of our knowledge, this is the first report on the ER retention of a tail-anchored protein. The carbohydrate chain of ALDH/OP3 was sensitive to endo H but insensitive to endo D, suggesting strongly that this chimera does not reach the cis Golgi. The ER retention of the chimera was checked further by treatment of transfected COS cells with brefeldin A, which effectively blocks membrane transport out of but not back to the ER (24). We have shown here that the carbohydrate chain of ALDH/OP3 is processed to an endo H-resistant form by cis/medial Golgi enzymes redistributed in the ER as a result of brefeldin A treatment. These biochemical results are consistent with morphological observations. Recently, we found that overexpression of msALDH in COS cells induced the formation of crystalloid ER (20), an aggregate of the smooth ER in a regular array. In addition, we showed that the smooth ER proliferated from the rough ER was transformed to the crystalloid ER and that the bulky cytoplasmic domain of msALDH was necessary for the formation of the crystalloid ER. By indirect immunofluorescence microscopy, we have shown here that ALDH/OP chimeras also form the crystalloid ER. Taken together, it appears that msALDH is retained in the ER through the blocking of the exit from the ER and that the smooth ER is assembled into the crystalloid ER through head-to-head association between the cytoplasmic domains of msALDH overexpressed on opposing membranes.

In summary, we have found that msALDH has a transmembrane topology and suggest that this protein is retained in the ER without recycling between the ER and the intermediate and cis Golgi compartments. Further investigation is required to elucidate the mechanisms for post-translational integration into the ER and for retention of msALDH in the ER.

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REFERENCES

1. Fuji-Kuriyama, Y., Negishi, M., Mikawa, R., and Tashiro, Y. (1979) J. Cell Biol. 81, 510–519
2. Bar-Nun, S., Kreibich, G., Adesnik, M., Alterman, L., Negishi, M., and Saba-tini, D. D. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 965–969
3. Sakaguchi, M., Mihara, K., and Sato, R. (1987) EMBO J. 6, 2425–2431
4. Morier, S., Van Luc, P., Kreibich, G., Sabatini, D. D., and Adesnik, M. (1988) J. Cell Biol. 107, 457–470
5. Szczesna-Skorupa, E., and Kemper, B. (1993) J. Biol. Chem. 268, 1757–1762
6. Shimozawa, O., Sakaguchi, M., Ogawa, H., Harada, N., Mihara, K., and Omura, T. (1993) J. Biol. Chem. 268, 21399–21402
7. Miyauchi, K., Masaki, R., Taketani, S., Yamamoto, A., Akayama, M., and Tashiro, Y. (1991) J. Biol. Chem. 260, 19536–19542
8. Takagi, Y., Ando, M., and Omura, T. (1985) J. Biochem. (Tokyo) 98, 1657–1662
9. Masaki, R., Yamamoto, A., and Tashiro, Y. (1994) J. Cell Biol. 126, 1407–1420
10. Pelham, H. R. B. (1988) EMBO J. 7, 913–918
11. Lewis, M. J., and Pelham, H. R. B. (1992) Cell 70, 535–364
12. Jackson, M. R., Nilsson, T., and Peterson, P. A. (1993) J. Cell Biol. 121, 317–333
13. Yamamoto, A., Masaki, R., and Tashiro, Y. (1985) J. Cell Biol. 101, 1733–1740
14. Murakami, M., Mihara, K., and Omura, T. (1994) J. Biochem. (Tokyo) 116, 164–175
15. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
16. Suenori, H., Kadodawa, Y., Goto, K., Araki, I., Kondoh, H., and Nakatsui, N. (1990) J. Cell. Biol. 29, 181–186
17. Kramer, W., and Frits, H.-J. (1987) Methods Enzymol. 154, 350–367
18. Lammli, U. K. (1970) Nature 227, 680–685
19. Kou, C. H., Yamagata, K., Miyaz, R. K., Bitsensky, M. W., and Miki, N. (1986) Mol. Brain Res. 1, 251–257
20. Yamamoto, A., Masaki, R., and Tashiro, Y. (1996) J. Cell. Biol. 139, 1727–1738
21. Robbins, P. W., Hubbard, S. C., Balch, W. E. (1987) Cell 50, 523–534
22. Misumi, Y., Misumi, Y., Miki, K., Takatsuki, A., Tamura, G., and Ikehara, Y.
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(1986) J. Biol. Chem. 261, 11398–11403
24. Lippincott-Schwartz, J., Yuan, L. C., Bonifadino, J. S., and Klausner, R. D. (1989) Cell 56, 801–813
25. Michikawa, T., Hamanaka, H., Otsu, H., Yamamoto, A., Miyawaki, A., Furuse, T., Tashiro, Y., and Mikoshiba, K. (1994) J. Biol. Chem. 269, 9184–9189
26. Kelleher, D. J., Kreibich, G., and Gilmore, R. (1992) Cell 69, 55–65
27. Nilsson, I., and von Heijne, G. (1993) J. Biol. Chem. 268, 5798–5801
28. Vergnes, G., Ramsden, J., and Waskell, L. (1995) J. Biol. Chem. 270, 3414–3422
29. Kutay, U., Ahnert-Hilger, G., Hartmann, E., Wiedenmann, B., and Rapoport, T. A. (1995) EMBO J. 14, 217–223
30. Inoue, A., Obata, K., and Akagawa, K. (1992) J. Biol. Chem. 267, 10613–10619
31. Ozols, J., and Heinemann, F. S. (1982) Biochim. Biophys. Acta 704, 163–173
32. Aalto, M. K., Ronne, H., and Keränen, S. (1993) EMBO J. 12, 4095–4104
33. von Heijne, G. (1996) EMBO J. 5, 3021–3027
34. Ahn, K., Szczesna-Skorupa, E., and Kemper, B. (1993) J. Biol. Chem. 268, 18726–18733
35. Szczesna-Skorupa, E., Ahn, K., Chen, C.-D., Doray, B., and Kemper, B. (1995) J. Biol. Chem. 270, 24327–24333
36. Südhof, T. C., Baumert, M., Perin, M. S., and Jahn, R. (1989) Neuron 2, 1475–1481
37. Hardwick, K. G., and Pelham, H. R. B. (1992) J. Cell Biol. 119, 513–521