The Juxtamembrane Sequence of Cytochrome P-450 2C1 Contains an Endoplasmic Reticulum Retention Signal*

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The N-terminal signal anchor of cytochrome P-450 2C1 mediates retention in the endoplasmic reticulum (ER) membrane of several reporter proteins. The same sequence fused to the C terminus of the extracellular domain of the epidermal growth factor receptor permits transport of the chimeric protein to the plasma membrane. In the N-terminal position, the ER retention function of this signal depends on the polarity of the hydrophobic domain and the signal KQS in the short hydrophilic linker immediately following the transmembrane domain. To determine what properties are required for the ER retention function of the signal anchor in a position other than the N terminus, the effect of mutations in the linker and hydrophobic domains on subcellular localization in COS1 cells of chimeric proteins with the P-450 signal anchor in an internal or C-terminal position was analyzed. For the C-terminal position, the signal anchor was fused to the end of the luminal domain of epidermal growth factor receptor, and green fluorescent protein was additionally fused at the C terminus of the signal anchor for the internal position. In these chimeras, the ER retention function of the signal anchor was rescued by deletion of three leucines at the C-terminal side of its hydrophobic domain; however, deletion of three valines from the N-terminal side did not affect transport to the cell surface. ER retention of the C-terminal deletion mutants was eliminated by substitution of alanines for glutamine and serine in the linker sequence. These data are consistent with a model in which the position of the linker sequence at the membrane surface, which is critical for ER retention, is dependent on the transmembrane domain.

Intracellular localization of secretory and membrane proteins results from the sequential action of sorting factors functioning at multiple steps, starting with insertion of the proteins into the membranes of the endoplasmic reticulum (ER).1 The transport from the ER of proteins destined for other cellular compartments was initially proposed to occur by bulk flow, that is, passive incorporation of ER contents into transport vesicles (1). The concentration of some proteins in the COPII transport vesicles that form at the ER is the same as that in the lumen of the ER (2), which is consistent with the bulk flow model (1). However, for other, perhaps most proteins, concentrations of the proteins are increased in the COPII transport vesicles, and the kinetics of transport are faster than expected for a bulk flow model, which indicates that exit from the ER is a selective process mediated by positive sorting signals (1, 3, 4). The sorting signals, by interaction with coat proteins or vesicle membrane proteins, mediate concentration of secretory and membrane proteins in selected regions of the ER, where they are packaged into the transport vesicles (5, 6). In the absence of sorting signals, proteins would not be concentrated in transport vesicles but could still be slowly transported from the ER by bulk flow unless other sorting signals or properties of the protein prevent their incorporation into the transport vesicle.

Primary determinants mediating localization of membrane proteins to the ER, Golgi, or plasma membrane have been mostly mapped to their TMDs (7–10). There is no obvious difference in the lengths or sequences of TMDs of ER and Golgi membrane proteins; however, it has been suggested that the length of the TMD is the main discriminatory factor between Golgi and plasma membrane proteins (9, 10). Consistent with the increasing thickness of the lipid bilayer along the secretory pathway, TMDs of the plasma membrane are longer than those of the Golgi. Moreover, for some ER membrane proteins, simple extension of the TMD was sufficient for targeting to the Golgi and plasma membrane (11, 12). In addition to length, both the distribution of hydrophobicity and polar residues in the TMD are also part of the sorting determinants (13–15).

Extramembranous sequences have been also found to strongly affect sorting of proteins to the ER, Golgi, or plasma membrane (16–20). In addition to the already mentioned positive sorting signals, these sequences may contain localization determinants that prevent transport to the next compartment, thus functioning as negative or true retention signals (20–22). This may be the mechanism used by some integral ER membrane proteins lacking any known sorting signals, which are excluded from export to the pre-Golgi compartment. Lack of transport from the ER as a consequence of incorporation of membrane proteins into large immobile networks has also been postulated (23, 24). However, this mechanism fails to explain the ER retention of proteins that have been shown to have high lateral mobility (25, 26). The sorting signals or properties of the proteins that prevent the incorporation of these mobile proteins into transport vesicles are not well understood. Either the proteins could be actively targeted to regions of the ER not involved in transport vesicle formation or some property of the protein could be incompatible with inclusion in the transport vesicle.

Cytochrome P-450 (P-450) 2C1/2 is inserted into the ER membrane via its N-terminal signal anchor sequence, which
functions as an ER retention sequence, independently of the ER retention also mediated by the catalytic, cytoplasmic domain (27). We have shown that the 28-amino acid signal anchor sequence of cytochrome P-450 2C1 prevents incorporation into the transport vesicles, resulting in static ER retention of either P-450 2C1 or chimeric proteins (15). Deletion of the 7-amino acid linker sequence following the TMD or mutation of the sequence $^{21}$KQS$^{23}$ in the linker resulted in chimeras that were no longer statically retained in the ER but were retained in the ER by retrieval from the intermediate compartment. Mutagenesis of the P-450 2C1 TMD indicated that ER retention mediated by the N-terminal 28 amino acids depended not only on its length but also on the distribution of the hydrophobic residues. These results suggest that the specific position or orientation of the TMD in the membrane determines whether the protein is incorporated into transport vesicles, possibly by properly positioning the linker sequence.

We have shown that the ER retention function of the signal anchor of cytochrome P-450 2C1 is dependent on its position in the protein. If the signal anchor was fused to the C terminus of the extracellular domain of EGFR or substituted for the TMD of EGFR, the resulting chimeric proteins were exported from the ER in transfected COS1 cells (27). If the large luminal domain in these chimeric proteins alters the position of the TMD in the membrane, then changing the length of the hydrophobic core or altering the distribution of the hydrophobicity might be able to restore the ER retention function. To test this hypothesis, we examined the effects of deletions of hydrophobic residues in the TMD and the effect of mutations in the linker sequence on the ER retention function of the signal anchor in an internal or C-terminal position. Our results demonstrate that in these positions, the native P-450 2C1 signal anchor does not mediate ER retention unless its TMD core is shortened in the C-terminal region, which may bring the linker residues KQS that are critical for ER retention closer to the lipid bilayer.

**EXPERIMENTAL PROCEDURES**

**Materials**—Tran$^{35}$S-label was from ICN Radiochemicals, endoglycosidase F was from New England Biolabs, the antibody against the extracellular domain of EGFR was from Upstate Biotechnology, rhodamine-conjugated goat anti-mouse antibody was from TAGO, Inc. (Burlingame, CA), mouse anti-OM10 antibody was from BD Biosciences, and anti-GFP antibody was from Roche Molecular Biochemicals. Cell culture media and antibiotics were from Life Technologies, Inc., and fetal bovine serum was from Gemini Bio-Products.

**Plasmid Constructions**—The construction of chimeras ECE (EGFR with its transmembrane domain replaced by the P-450 2C1 signal anchor) and ECO (ECE with the cytoplasmic domain of EGFR deleted) in the pCMV vector has been described (27). To construct chimeras EC/GFP, in which the GFP coding sequence is attached to the C terminus of the P-450 2C1 signal anchor, plasmid ECC (27) was digested with BglII and HindIII, and the obtained fragment was inserted into the BglIII-HindIII-digested vector pEGFP-N1. All mutations of the P-450 2C1 N-terminal signal anchor were prepared by polymerase chain reaction using a set of designed primers and EcoRI or EcoII DNA as a template. Construction of a plasmid encoding a glycosylation tag at the C terminus of GFP and of chimera C1/GFP (C1(1–28)/GFP) was described previously (28). This could be the result either of the overall shortening of the TMD or of a change in hydrophobicity of its C-terminal region. To distinguish between these possibilities, the three Val at positions 4–6 at the N-terminal side of the TMD (Fig. 1) were deleted. In contrast to the C-terminal deletion, the carbohydrate side chains of the N-terminal mutant were resistant to endo H digestion (Fig. 2A), as in ECO, so that the signal anchor with three valines deleted did not regain an ER retention function. These results suggest that the specific distribution of the hydrophobic residues in the TMD is an important determinant for ER retention.

In the ECO context the P-450 2C1 signal anchor is at the C terminus of the chimeric protein, whereas in ECE, which is also transported from the ER, it is followed by a large cytoplasmic domain of EGFR that could potentially contain positive transport signals. To examine whether the characteristics of the signal anchor were similar in an internal position when flanked by a linker that normally is not localized to the plasma membrane, ECO and its deletion mutants were fused to the N terminus of GFP. The localization of these fluorescent proteins in the cells was consistent with the studies with ECO, EC/GFP and ECO(−3V)/GFP were transported out of the ER as indicated by an intracellular fluorescence pattern different from ER localization (compare with Fig. 4A, C1/GFP) and by the presence
of fluorescence at the surface of the cells (Fig. 2B, left panels). The location of the proteins at the surface of the cell was supported by the detection of the chimera (as well as endogenous EGFR) on the surface of unpermeabilized cells by an antibody against extracellular domain of EGFR on the surface of the cells (Fig. 2B, right panel). In contrast, cells expressing EC(-3L)/GFP had a reticular cytoplasmic pattern characteristic of ER localization (Fig. 2B, left panel) that was clearly different from the surface localization of EGFR (Fig. 2B, right panel). The presence of GFP did not affect the distribution of the proteins in the cell because the endoglycosidase H sensitivity of the GFP chimeras was the same as that of the corresponding proteins without GFP (data not shown). In both the C-terminal and internal positions, therefore, deletions on the C-terminal side but not the N-terminal side, of the TMD resulted in a gain of ER retention function. These results are consistent with previous studies of the signal anchor in its normal N-terminal position, in which mutations in the C-terminal side of the TMD had the greatest effect on ER retention function (15).

The Role of the Linker Sequence in ER Retention Mediated by an Internally Located Signal Anchor—Studies on the signal anchor in its normal N-terminal position showed that both the TMD and the linker region contribute to its ER retention function (15). Specific mutations of the sequence KQS in the linker interfered with ER retention, whereas changes in the distribution and number of hydrophobic residues primarily in the C-terminal half of the TMD blocked retention (15). One interpretation of these results was that the orientation or position of the linker sequence or the C-terminal half of the TMD relative to the membrane was critical for ER retention. If the mechanism for ER retention mediated by the signal anchor in an internal or C-terminal position is the same as when it is in the N-terminal region, then the KQS sequence in the linker should also be important for retention mediated by the C-terminal or internal signal anchor.

Our previous studies with chimeras containing an N-terminally positioned signal anchor showed that mutation of both Lys21 and Ser23 (K21N and S23V) in the linker largely eliminated static ER retention. We therefore analyzed the effect of mutating these residues in chimera EC(-3L)/GFP. Surprisingly, the carbohydrate side chains of this mutant, EC(-3L)/NQV/GFP, remained sensitive to endo H digestion (Fig. 3A). Consistent with this observation, the fluorescence of this mutant was not localized to the plasma membrane; however, the distribution was not typical of ER localization either (Fig. 3B, left panel). Instead, the fluorescence was localized in a perinuclear region, which indicates that the protein was not retained efficiently in the ER. The pattern of GFP fluorescence suggests that the protein is transported to the Golgi. The distribution of fluorescence is similar to that of the cis-Golgi marker protein GM130 (30), which was detected by its antibody and visualized with rhodamine-conjugated secondary antibody (Fig. 3B, right panel). The sensitivity of the carbohydrate side chains to cleavage with endo H suggested that the mutant was transported no further than the cis-Golgi (31).

To further examine the importance of the KQS linker sequence, we analyzed the effect on subcellular localization of substituting three alanines for KQS (EC(-3L)/KAA/GFP) (Fig. 1). In contrast to EC(-3L)/GFP, significant resistance to endo H digestion of the carbohydrate side chains was observed for this mutant, which indicates that it is not efficiently retained in the ER (Fig. 3A). Furthermore, the distribution of the fluorescence in cells expressing the EC(-3L)/AAA/GFP chimera, which includes fluorescence at the surface of the cells, indicates that the protein is not retained in the ER but is transported to the cell surface (Fig. 3B, left panel). To address the possibility that this effect is caused by the elimination of the positively charged Lys21, which effectively extends the TMD by three amino acids, we also tested a mutant with only residues 22 and 23 (QS) substituted by alanines. This mutant, EC(-3L)/KAA/GFP, is also transported from the ER, as shown by its resistance to endo H digestion (Fig. 3A) and the presence of the fluorescent protein in the plasma membrane (Fig. 3B, left panel). For both EC(-3L)/AAA/GFP and EC(-3L)/KAA/GFP, detection of the extracellular domain of EGFR on the surface of the cells provides further evidence that the chimeras are transported from the ER to the plasma membrane (Fig. 3B, right panel). The requirement for the linker sequence KQS for ER retention when the (-3L) signal anchor is present at the C terminus or internally indicates that the mechanism for retention is the same when the signal anchor is present in these positions as when it is present at the N terminus.

Effects of Shortening the TMD on Targeting by the N-terminally Located P-450 2C1 Signal Anchor—In previous studies on the requirements for the ER retention mediated by the
mutations in the linker sequence.

The GFP was consistent with an ER localization (Fig. 4A). In agreement with the N-terminally located signal anchor. In general, mutations in the C-terminal half of the TMD had greater effects. In the linker sequence, the specific sequence KQS was important for static ER retention. The more specific sequence requirement in the linker suggested that this sequence might interact with other proteins. These results led to the idea that the orientation and position of the C-terminal portion of the TMD and the linker sequence relative to the membrane were important for ER retention. When the P-450 2C1 signal anchor is placed in an internal or C-terminal position, its ER retention function is lost. In terms of the proposed model, a large luminal domain may alter the position of the TMD in the membrane, which in turn affects the position of the KQS sequence. The size of the luminal domain may be important because fusion of a smaller 29-amino acid glycosylation tag sequence to the N terminus did not affect ER retention (15). Such mutations in the TMD were relatively nonspecific with regard to the sequence but altered the length of the hydrophobic core or the distribution of hydrophobic residues. In general, mutations in the C-terminal half of the TMD had greater effects. In the linker sequence, the specific sequence KQS was important for static ER retention. The more specific sequence requirement in the linker suggested that this sequence might interact with other proteins. These results led to the idea that the orientation and position of the C-terminal portion of the TMD and the linker sequence relative to the membrane were important for ER retention. When the P-450 2C1 signal anchor is placed in an internal or C-terminal position, its ER retention function is lost. In terms of the proposed model, a large luminal domain may alter the position of the TMD in the membrane, which in turn affects the position of the KQS sequence.

FIG. 3. Subcellular localization of chimeric proteins carrying mutations in the linker sequence. A COS1 cells were transfected with chimeras EC/−3L/AAA/GFP (AAA), EC/−3L/KAA/GFP (KAA), and EC/−3L/NQV/GFP (NQV), and 48 h later cells were processed for endo H (EH) sensitivity as described in the legend to Fig. 2A. B COS1 cells transfected with the same chimeras as in A were fixed, and unpermeabilized cells were immunostained with an antibody against the extracellular domain of EGFR (AAA and KAA), or permeabilized cells were immunostained with mouse antibody to the cis-Golgi marker protein GM130 (NQV), followed in each case by rhodamine-conjugated secondary antibody. Fluorescence from GFP is shown in the left panels, and that from rhodamine in the right panels.

P-450 2C1 signal anchor in its normal N-terminal position, we demonstrated that lengthening the TMD resulted in a loss of ER retention, but mutations that shortened the TMD were not studied (15). Because deletion of three leucines had dramatic effects on signal anchor retention function when the signal anchor was C-terminal or internal, whereas deletion of three valines did not, we examined the effects of these mutations on the N-terminally located signal anchor. In agreement with previous studies (15, 25), the fluorescent distribution of C1/GFP was consistent with an ER localization (Fig. 4A). The deletion of either three leucines or three valines, however, altered the targeting function of the signal anchor, and the proteins were no longer inserted as type 1 membrane proteins, so that the effect on ER retention could not be determined. The diffuse pattern observed with C1(−3L)/GFP is consistent with a cytoplasmic distribution (Fig. 4A), which indicates that this deletion inhibited the targeting of the protein to the ER.

The C1(−3V)/GFP mutant exhibited a punctate pattern of fluorescence, suggesting that it had been translocated across the ER membrane and transported through the secretory pathway to transport vesicles. Several observations supported this conclusion. First, treatment of the cells with brefeldin A, which prevents forward transport from the ER and leads to retrograde transport of Golgi proteins to the ER, resulted in a reticular pattern of fluorescence consistent with ER retention (not shown). Second, after radiolabeling and a prolonged chase, small amounts of protein immunoreactive to GFP antisera are present in the medium of transfected cells (Fig. 4B). Similar amounts of the protein are present in the medium of cells transfected with a chimera containing the secretory signal sequence of parathyroid hormone fused to GFP, PTH/GFP (Fig. 4B). Finally, a 29-amino acid glycosylation tag was placed at the C terminus of C1(−3V)/GFP and PTH/GFP. Glycosylation of the tag would indicate that the protein was translocated across the ER membrane. In both cases, a fraction of the radiolabeled protein had a slower electrophoretic mobility on SDS-polyacrylamide gels, suggesting that it had been glycosylated (Fig. 4C). This was confirmed by the elimination of the slower mobility protein by treatment with N-glycosidase F, which removes carbohydrate side chains. Thus, whereas deletion of three leucines or three valines in the TMD of the P-450 signal anchor does not affect its stop transfer and membrane anchor properties when the TMD is internal or C-terminally located, the same mutations in the N-terminally located signal anchor either inhibit ER targeting (−3L) or convert it to a translocation signal that is presumably cleaved (−3V).

DISCUSSION

Mutations in either the TMD or the following linker sequence can interfere with the static ER retention function of the P-450 2C1 signal anchor sequence when in its normal N-terminal location (15). Such mutations in the TMD were relatively nonspecific with regard to the sequence but altered the length of the hydrophobic core or the distribution of hydrophobic residues. In general, mutations in the C-terminal half of the TMD had greater effects. In the linker sequence, the specific sequence KQS was important for static ER retention. The more specific sequence requirement in the linker suggested that this sequence might interact with other proteins. These results led to the idea that the orientation and position of the C-terminal portion of the TMD and the linker sequence relative to the membrane were important for ER retention. When the P-450 2C1 signal anchor is placed in an internal or C-terminal position, its ER retention function is lost. In terms of the proposed model, a large luminal domain may alter the position of the TMD in the membrane, which in turn affects the position of the KQS sequence. The size of the luminal domain may be important because fusion of a smaller 29-amino acid glycosylation tag sequence to the N terminus did not affect ER retention (15). This idea is supported by the observation that reducing the length of the C terminally or internally positioned TMD by deletions in its C-terminal portion restored ER retention function. This dependence on length is similar to the observation that ER retention of cytochrome b5 was lost when its C-terminal TMD was lengthened (11). The inability of the mutant with deletion of three valines from the N-terminal portion of the signal anchor to restore ER retention is more difficult to explain from this model but may indicate that the length of the TMD is less important than its orientation, i.e. the degree of slant in the membrane.

Because the TMD requirements for ER retention are different depending on the position of the signal anchor, it is possible that the mechanisms for ER retention are different. If different mechanisms are involved, then the KQS sequence in the linker region might not be as important for the ER retention function of the signal anchor in a C-terminal or internal position as it is in the N-terminally located signal anchor. However, substitution of Ala either for KQS or QS or substitution of NQV for KQS resulted in transport from the ER of chimeras containing the signal anchor in an internal or C-terminal position. The requirement for the KQS sequence regardless of position suggests that the mechanisms are the same and that both the
TMD and the juxtamembrane linker sequence are required for ER retention.

Sorting determinants for transmembrane proteins have been identified in juxtamembrane sequences on both the cytosolic and luminal sides of the membrane. Positive sorting signals in the cytoplasmic tails of membrane proteins contribute to targeting in the secretory pathway and endocytosis and to basolateral membranes in polarized cells (reviewed in Ref. 6). Similarly, a short juxtamembrane domain mediates the sorting of MAL to specific membrane microdomains (32). A negative cytoplasmic retention signal is required for localization of Golgi proteins (19, 20) and is present in the catalytic domain of P-450 2C2 (27). Luminal or extramembranous sequences functioning in ER retention have also been found in hepatitis C virus glycoprotein E1 (33), asialoglycoprotein (34), hepatitis B virus glycoprotein (35), aldehyde dehydrogenase (36), and some reporter proteins in the protozoan parasite Toxoplasma gondii (37). A conserved sequence motif that mediates the targeting has not been identified in any of these cases.

The KQS motif identified in our studies as being required for the ER retention function of the P-450 2C1 signal anchor has not been described previously as a sorting signal. This sequence is not highly conserved in P-450s. KQS and similar sequences (RQS and RQV) are present in many P-450 2C subfamily members and in the most closely related subfamily, P-450 2E (KQI and RQV) but not in most other P-450s. In human P-450 2E1, which has an RQV sequence, the signal anchor does not mediate static ER retention (22). ER retention, therefore, appears to be mediated by different mechanisms for different P-450s. Whether the use of the KQS motif as a retention signal in P-450 2C proteins is related to a unique physiological function is not known.

Deletions within the hydrophobic core of the P-450 2C1 signal anchor, when in its normal N-terminal position, had dramatically different effects on its targeting function depending on whether the N- or C-terminal portion of the TMD was shortened. These results agree with earlier observations of Sato et al. (38), who showed that for the signal anchor of rabbit P-450 IA1, N-terminal deletions caused translocation and processing of the protein, whereas C-terminal deletions eliminated interaction with the membrane resulting in a cytosolic protein. These data are consistent with the loop model of signal sequence insertion, in which the C terminus of the signal enters the membrane first, so that its hydrophobicity may be more critical for membrane interaction (39). Different results were obtained with the signal anchor of cytochrome P-450 M1. This signal anchor mediated ER translocation with either N-terminal or C-terminal deletions, which probably reflects different sequences of these signal anchors or the systems used: cell-free translation-translocation versus transfected cells (38).

The loss of ER retention function of the P-450 2C1 signal anchor in a C-terminal position differs from the continued ER retention function mediated by the 21-amino acid TMD of the closely related P-450 M1 signal anchor fused to the C terminus of carboxylesterase (40). The differences in the two experiments may be related to the nature of the luminal domain, which could have different effects on the signal anchor sequence. For instance, in terms of the model described above, carboxylesterase may perturb the membrane position of the P-450 TMD less than the luminal domain of EGFR. Surprisingly, deletion of 15 of the 21 amino acids of the M1 TMD, leaving only four core hydrophobic residues, did not affect its ER retention function (40). An alternative explanation for the different results might be that the mechanism for ER retention in this fusion protein is unrelated to the normal mechanism mediated by the signal anchor in the N-terminal position.

TMDs play a critical role as sorting signals for multiple organelles. The targeting may result from preference of a TMD for membranes with specific lipid compositions or thickness or by more specific interactions with lipids or helices of other membrane proteins. The distribution of polar residues in TMDs has been shown to be important for sorting, which suggests that interactions with other proteins may be involved. In addition to these direct interactions of the TMD with membrane components, the TMD may also determine the position relative to the membrane of juxtamembrane sequences, such as the KQS motif described in this paper, which is critical for proper sorting. In this case, the TMD would be a relatively passive anchor, whereas the juxtamembrane sequence would be the primary signal for sorting.

REFERENCES
1. Rothman, J. E., and Orci, L. (1992) Nature 355, 409–415
2. Martinez-Menarguez, J. A., Geuze, H. J., Slot, J. W., and Klumperman, J. (1999) Cell 98, 81–90
3. Nishimura, N., and Balch, W. E. (1997) Science 277, 556–558
4. Kuehn, M. J., Herrmann, J. M., and Schekman, R. (1998) Nature 391, 187–191
5. Mellman, I., and Warren, G. (2000) Cell 100, 99–112
6. Rothman, J. E., and Wieland, F. T. (1996) Science **272**, 227–234
7. Letourneur, F., and Cosson, P. (1998) *J. Biol. Chem.* **273**, 33273–33278
8. Rayner, J. C., and Pelham, H. R. (1997) *EMBO J.* **16**, 1832–1841
9. Bretscher, M. S., and Munro, S. (1993) *Science* **261**, 1280–1281
10. Munro, S. (1995) *EMBO J.* **14**, 4695–4704
11. Pedrazzini, E., Villa, A., and Borgese, N. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 4207–4212
12. Yang, M., Ellenberg, J., Bonifacino, J., and Weissman, A. (1997) *J. Biol. Chem.* **272**, 1970–1975
13. Bretscher, M. S., and Munro, S. (1993) *Science* **261**, 1280–1281
14. Pedrazzini, E., Villa, A., and Borgese, N. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 4207–4212
15. Yang, M., Ellenberg, J., Bonifacino, J., and Weissman, A. (1997) *J. Biol. Chem.* **272**, 1970–1975
16. Szczesna-Skorupa, E., Chen, C.-D., Doray, B., and Kemper, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14793–14798
17. Li, Y., Smith, T., Grabski, S., and DeWitt, D. L. (1998) *J. Biol. Chem.* **273**, 29830–29837
18. Szczesna-Skorupa, E., Ahn, K., Chen, C.-D., Doray, B., and Kemper, B. (1999) *J. Biol. Chem.* **274**, 24327–24333
19. Mead, D. A., Szczesna-Skorupa, E., and Kemper, B. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 67–74
20. Szczesna-Skorupa, E., Chen, C., Rogers, S., and Kemper, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14793–14798
21. Li, Y., Smith, T., Grabski, S., and DeWitt, D. L. (1998) *J. Biol. Chem.* **273**, 29830–29837
22. Szczesna-Skorupa, E., Chen, C., and Kemper, B. (1995) *J. Biol. Chem.* **270**, 24327–24333
23. Gaynor, E. C., te Heesen, S., Graham, T. R., Aebi, M., and Emr, S. D. (1994) *J. Cell Biol.* **127**, 653–665
24. Ivessa, N. E., De Lemos-Ciarandini, C., Tsao, Y. S., Takatsuki, A., Adessnik, M., Sabatini, D. D., and Kreibich, G. (1992) *J. Cell Biol.* **117**, 949–958
25. Szczesna-Skorupa, E., Chen, C., Rogers, S., and Kemper, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14793–14798
26. Li, Y., Smith, T., Grabski, S., and DeWitt, D. L. (1998) *J. Biol. Chem.* **273**, 29830–29837
27. Szczesna-Skorupa, E., Ahn, K., Chen, C.-D., Doray, B., and Kemper, B. (1999) *J. Biol. Chem.* **274**, 24327–24333
28. Mead, D. A., Szczesna-Skorupa, E., and Kemper, B. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 67–74
29. Szczesna-Skorupa, E., Chen, C., Rogers, S., and Kemper, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14793–14798
30. Li, Y., Smith, T., Grabski, S., and DeWitt, D. L. (1998) *J. Biol. Chem.* **273**, 29830–29837
31. Szczesna-Skorupa, E., Ahn, K., Chen, C.-D., Doray, B., and Kemper, B. (1999) *J. Biol. Chem.* **274**, 24327–24333
32. Mead, D. A., Szczesna-Skorupa, E., and Kemper, B. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 67–74
33. Szczesna-Skorupa, E., Chen, C., and Kemper, B. (1993) *J. Biol. Chem.* **268**, 1757–1762
34. Nakamura, N., Lowe, M., Levine, T. P., Rabouille, C., and Warren, G. (1997) *Cell* **89**, 445–455
35. Kornfeld, R., and Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54**, 631–664
36. Puertollano, R., and Alonso, M. A. (1998) *J. Biol. Chem.* **273**, 12740–12745
37. Mottola, G., Jourdan, N., Castaldo, G., Malagolini, N., Lahm, A., Serafi –Cesì, F., Migliaccio, G., and Bonatti, S. (2000) *J. Biol. Chem.* **275**, 24070–24079
38. Tolchinsky, S., Yuk, M. H., Ayalon, M., Lodish, H. F., and Lederkremer, G. Z. (1996) *J. Biol. Chem.* **271**, 14496–14503
39. Kuroki, K., Russnak, R., and Ganem, D. (1989) *Virology* **170**, 445–4466
40. Masaki, R., Yamamoto, A., and Tashiro, Y. (1994) *J. Cell Biol.* **126**, 1407–1420
41. Hoppe, H. C., and Joiner, K. A. (2000) *Cell Microbiol.* **2**, 569–578
42. Sato, T., Sakaguchi, M., Mihara, K., and Omura, T. (1990) *EMBO J.* **9**, 2391–2397
43. Shaw, A. S., Rottier, P. J., and Rose, J. K. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 7592–7596
44. Murakami, K., Mihara, K., and Omura, T. (1994) *J. Biochem.* (Tokyo) **116**, 164–175
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