Biosynthesis of the Human Transferrin Receptor in Cultured Cells*

M. Bishr Omary‡ and Ian S. Trowbridge
From the Department of Cancer Biology, The Salk Institute, San Diego, California 92128

The biosynthesis and degradation of the cell surface transferrin receptor has been investigated. The receptor is a glycoprotein, and evidence is presented that the mature receptor contains both complex and high mannosone \(N\)-asparagine-linked oligosaccharides that are synthesized via a common high mannosone intermediate as previously described for other glycoproteins. It is shown that fatty acid is associated with only the mature form of the receptor and that addition of fatty acid to the receptor can occur as long as 48 h after synthesis. Glycosylation is not an absolute requirement for the receptor to act as acceptor for fatty acid, nor for transport to the cell surface, although the efficiency of both processes may be reduced in tunicamycin-treated cells. The protein moiety of the transferrin receptor is degraded with a half-life of approximately 60 h.

Membrane receptors constitute a diverse class of molecules involved in the binding of various hormones, growth factors, and metabolites necessary for normal cell growth and function (1-3). The cell surface receptor for transferrin binds this serum iron-transport protein (4-7) and is believed to serve an essential role in the transport of iron across the plasma membrane (8). Transferrin receptors were initially identified on maturing erythroid cells and placenta (9, 10). More recently, it has been shown that transferrin receptors are found in abundance on various types of proliferating cells and may be a useful surface marker for both normal stem cells and tumor cells (11-14). The transferrin receptor is a glycoprotein consisting of two similar disulphide-bonded subunits (M, ~ 95,000), and monoclonal antibodies against the receptor found on human cells have been obtained (15-18).

Here we have studied the sequence of events that occurs during the biosynthesis of the receptor, including covalent attachment of fatty acid (19), and provide an estimate of its rate of turnover in the plasma membrane.

MATERIALS AND METHODS

Cell Lines—CCRF-CEM cells, a human leukemic T-cell line, were used (20). Cells were grown in RPMI 1640 medium supplemented with 10% horse serum and 10 \(\mu\)M 2-mercaptoethanol. Chemicals—TPCK-trypsin and glucose oxidase were purchased from Worthington. Lactoperoxidase and tunicamycin were obtained from Calbiochem-Behring Corp., ovomucoid trypsin inhibitor and sodium deoxycholate were from Sigma, SDS was from BDH Chemicals Ltd., and Nonidet P-40 was from Shell Chemical. For radiolabeled chemicals, \(\mathrm{L}^{[\mathrm{35S}]}\)methionine (1,190 Ci/mmol) was purchased from Amersham, and [\(\mathrm{3H}\)]palmitic acid (11.8 Ci/mmol) was obtained from New England Nuclear.

Radiolabeling Procedures—Cells incubated with or without tunicamycin (4-5 \(\mu\)g/ml) were labeled with [\(\mathrm{3H}\)]palmitic acid or [\(\mathrm{35S}\)]methionine. Tunicamycin dilutions were obtained from a frozen stock solution (5 mg/ml in dimethyl sulfoxide DMSO) and incubation was for 90 min prior to labeling. For [\(\mathrm{3H}\)]palmitic acid labeling, [\(\mathrm{3H}\)]palmitic acid in benzene was dried and then redissolved in 80% (v/v) ethanol/H\(_2\)O. Labeled palmitate was added to cell suspensions such that the ethanol concentration did not exceed 0.3%. Cells (4 \(\times\) 10\(^7\) cells/ml) were labeled with 100 \(\mu\)Ci of [\(\mathrm{3H}\)]palmitic acid/ml in RPMI 1640 medium supplemented with 5% fetal calf serum dialyzed against PBS.

Membrane receptors constitute a diverse class of molecules involved in the binding of various hormones, growth factors, and metabolites necessary for normal cell growth and function (1-3). The cell surface receptor for transferrin binds this serum iron-transport protein (4-7) and is believed to serve an essential role in the transport of iron across the plasma membrane (8). Transferrin receptors were initially identified on maturing erythroid cells and placenta (9,10). More recently, it has been shown that transferrin receptors are found in abundance on various types of proliferating cells and may be a useful surface marker for both normal stem cells and tumor cells (11-14). The transferrin receptor is a glycoprotein consisting of two similar disulphide-bonded subunits (M, ~ 95,000), and monoclonal antibodies against the receptor found on human cells have been obtained (15-18).

Here we have studied the sequence of events that occurs during the biosynthesis of the receptor, including covalent attachment of fatty acid (19), and provide an estimate of its rate of turnover in the plasma membrane.

MATERIALS AND METHODS

Cell Lines—CCRF-CEM cells, a human leukemic T-cell line, were used (20). Cells were grown in RPMI 1640 medium supplemented with 10% horse serum and 10 \(\mu\)M 2-mercaptoethanol. Chemicals—TPCK-trypsin and glucose oxidase were purchased from Worthington. Lactoperoxidase and tunicamycin were obtained from Calbiochem-Behring Corp., ovomucoid trypsin inhibitor and sodium deoxycholate were from Sigma, SDS was from BDH Chemicals Ltd., and Nonidet P-40 was from Shell Chemical. For radiolabeled chemicals, \(\mathrm{L}^{[\mathrm{35S}]}\)methionine (1,190 Ci/mmol) was purchased from Amersham, and [\(\mathrm{3H}\)]palmitic acid (11.8 Ci/mmol) was obtained from New England Nuclear.

Radiolabeling Procedures—Cells incubated with or without tunicamycin (4-5 \(\mu\)g/ml) were labeled with [\(\mathrm{3H}\)]palmitic acid or [\(\mathrm{35S}\)]methionine. Tunicamycin dilutions were obtained from a frozen stock solution (5 mg/ml in dimethyl sulfoxide DMSO) and incubation was for 90 min prior to labeling. For [\(\mathrm{3H}\)]palmitic acid labeling, [\(\mathrm{3H}\)]palmitic acid in benzene was dried and then redissolved in 80% (v/v) ethanol/H\(_2\)O. Labeled palmitate was added to cell suspensions such that the ethanol concentration did not exceed 0.3%. Cells (4 \(\times\) 10\(^7\) cells/ml) were labeled with 100 \(\mu\)Ci of [\(\mathrm{3H}\)]palmitic acid/ml in RPMI 1640 medium supplemented with 5% fetal calf serum dialyzed against PBS.

Membrane labeling of cells with [\(\mathrm{35S}\)]methionine was carried out in methionine-free RPMI 1640 medium containing 5% dialyzed fetal calf serum. For endo H experiments, cells (6 \(\times\) 10\(^7\) cells/ml) were pulse-labeled with [\(\mathrm{35S}\)]methionine for 10 min using 150-200 \(\mu\)Ci/ml (0.6 ml total volume), then diluted with 0.4 ml of RPMI 1640 medium supplemented with 10 mM L-methionine and 10% fetal calf serum. For the turnover rate of [\(\mathrm{3H}\)]palmitate- and [\(\mathrm{35S}\)]methionine-labeled transferring receptor, labeled cells were washed twice using RPMI 1640 medium supplemented with 10% horse serum, 10 \(\mu\)M 2-mercaptoethanol, and 100 \(\mu\)M palmitic acid, then incubated in the same medium for various chase periods.

Immunological Procedures—Monoclonal anti-transferrin receptor antibody (B3/25) has been described previously (15, 16) and ascitic fluid was used for immunoprecipitation. To obtain immunoprecipitates, labeled cells were lysed with 1% Nonidet P-40 in PBS (1-2 \(\times\) 10\(^7\) cells/ml). After 20 min at 4 \(\circ\)C, nuclei were removed by centrifugation for 2 min in an Eppendorf model 3200 microcentrifuge. The lysates were then mixed with a solution containing 10% Nonidet P-40, 10% sodium deoxycholate, and 1% SDS in PBS (101, v/v; lysate: detergent solution), followed by the addition of 60 \(\mu\)l of 12% (v/v) formaldehyde-fixed Staphylococcus aureus (21) per ml of lysate. After 20 min at 4 \(\circ\)C, the bacteria were removed by centrifugation at 20,000 \(\times\) \(g\) for 60 min. Antibody complexes were formed by incubating samples of the cell extract with the B3/25 monoclonal antibody for 15 min, then with goat anti-mouse IgG for an additional 15 min. Immune complexes were then collected using a 1% (v/v) suspension of S. aureus (150 \(\mu\)l of bacteria suspension/extracts from 1-2 \(\times\) 10\(^7\) cells) and washed 3 times with 0.4 ml of 0.5% deoxycholate, 0.5% Nonidet P-40, 0.05% SDS in PBS. The antigens were released from the S. aureus bacteria by boiling for 2 min in electrophoresis sample buffer (22). Control immunoprecipitates for nonspecific binding were similarly prepared except that the monoclonal B3/25 antibody was not added.

Trypsin Digestion—Labeled cells (1-2 \(\times\) 10\(^7\) cells/ml) were digested with TPCK-trypsin (256 units/mg, 50 \(\mu\)g/ml) at 23 \(\circ\)C in a total volume of 150 \(\mu\)l. After 15 min, proteolysis was terminated by adding 100 \(\mu\)l of 5 mg/ml of ovomucoid trypsin inhibitor. Supernatants were removed after centrifugation of the cells in a microcentrifuge for 1 min. Immunoprecipitates were then prepared from either the supernatants or from the remaining cells after washing once with 0.6 ml of PBS.

Treatment with Endo-\(\beta\)-N-acetylgalactosaminidase H—Endo H was a kind gift from Dr. Phillips Robbins, Massachusetts Institute of Technology. S. aureus immunoprecipitates (three for each time point) were treated and washed as described above. An additional washing step was carried out using 0.4 ml of 10 mM Tris-HCl containing 0.1% Nonidet P-40 (pH 7.5). The procedure used for endo H digestion was similar to that described by Rothenberg and Boyse (23) except that the incubation was for 15-18 h at 37 \(\circ\)C. One of the three immunoprecipitates was stored at 20 \(\circ\)C and the remaining two were incu-
bated at 37 °C in the presence or absence of endo H (5–10 μg/ml). No differences were observed upon SDS-polyacrylamide gel analysis between samples stored at −20 °C and those incubated at 37 °C without endo H.

Miscellaneous Methods—SDS-polyacrylamide gel electrophoresis was carried out as described previously (24) using 7.5 or 10% polyacrylamide gels. Processing of gels for fluorography was as described by Bonner and Laskey (25). Protein synthesis was estimated from the amount of radioactivity incorporated into trichloroacetic acid-precipitable, ethanol-insoluble material obtained from 1% Nonidet P-40 extracts of labeled cells. Gels were scanned using a Zeineh scanning densitometer (Biomed Instruments Inc.).

RESULTS

Glycosylation of the Transferrin Receptor—The biosynthesis of the transferrin receptor in the human leukemic cell line CCRF-CEM was studied using the monoclonal antibody designated B3/25 to isolate the receptor by immunoprecipitation. As shown in Fig. 1, newly synthesized receptor detected by labeling cells for 10 min with [35S]methionine had an apparent molecular weight of 88,000 (Fig. 1a). Within 4 h, this species was converted into a Mr = 95,000 species (Fig. 1i) indistinguishable in mobility on SDS-polyacrylamide gel electrophoresis from the mature form of the cell surface receptor accessible to labeling by lactoperoxidase-catalyzed iodination. Digestion of the [35S]methionine-labeled receptor with endo H (26, 27) converted the Mr = 88,000 species to 80,000 (Fig. 1u), while the apparent molecular weight of the mature receptor was reduced from 95,000 to 89,000 (Fig. 1, i and j).

Similar experiments with receptors labeled with [2-3H]mannose showed that both the immature and mature receptors contained mannose, but endo H digestion of the immature Mr = 88,000 species removed all labeled mannose residues while only partial loss of mannose occurred during digestion of the mature Mr = 95,000 receptor (data not shown). The interpretation of these results is that as early as 10 min after synthesis, the transferrin receptor is glycosylated and the oligosaccharides initially present are all of the high mannose type. Subsequently some, but not all, of these oligosaccharides are converted to complex oligosaccharides. Glycopeptide analysis by gel filtration of the receptor metabolically labeled with either mannose, galactose, or glucosamine was also consistent with the presence of both complex and high mannose oligosaccharides on the mature receptor.

Glycosylation of the transferrin receptor was also studied using the antibiotic tunicamycin, which inhibits asparagine-linked glycosylation by blocking the formation of the donor lipid-linked oligosaccharide (28, 29). As shown in Fig. 2c, a major radiolabeled species with a molecular weight of approximately 79,000 could be detected after incubating CCRF-CEM cells with tunicamycin (5 μg/ml). Under these conditions, none of the normally glycosylated species (Fig. 2b) was found and overall protein synthesis was not affected, whereas incorporation of [2-3H]mannose into cellular glycoproteins was inhibited by more than 90%. A minor species (Mr = 74,000) also appeared to be specifically immunoprecipitated from tunicamycin-treated cells. Some of the unglycosylated transferrin receptors could be transported to the cell surface, since trypsin treatment of intact tunicamycin-treated cells resulted in the release of a tryptic fragment of the receptor into the supernatant (Fig. 2f). Under the same conditions, pulse-labeled (5 min) intracellular receptor was not cleaved by trypsin, indicating that under the conditions used, transferrin receptors at an intracellular location were not sensitive to proteolysis (data not shown). However, assuming that the efficiency of immunoprecipitation of the unglycosylated receptor and its tryptic fragment are identical with their glycosylated counterparts, then it is evident from the data presented in Fig. 2 that a much smaller fraction of the unglycosylated receptor than glycosylated receptor reached the cell surface during the chase period (12 versus 58%).

Palmitate Is Associated with only the Mature Form of the Transferrin Receptor—We have previously shown that the transferrin receptor can be labeled with [3H]palmitate (19). Since the immature and mature forms of the transferrin
Transferrin Receptor Biosynthesis

receptor could be distinguished by their migration on SDS-polyacrylamide gel electrophoresis and susceptibility to endoglycosidase-H digestion, it was possible to investigate when, during biosynthesis, fatty acid becomes associated with the receptor. As shown in Fig. 3, even when cells were briefly labeled with [3H]palmitate (10 min), only the mature form of the transferrin receptor incorporated detectable amounts of radioactivity (Fig. 3, compare tracks a-f with g-j). This observation then raised the question of whether fatty acid becomes bound to the receptor at a defined late stage in its biosynthesis after oligosaccharide processing is complete or whether the mature form of the transferrin receptor can be labeled with [3H]palmitate at any time subsequent to its insertion into the plasma membrane. This point was investigated by incubating CCRF-CEM cells in the presence of tunicamycin for various periods of time, then labeling cells with [3H]palmitate. Transferrin receptors synthesized in tunicamycin-treated cells could be distinguished from receptors that were made before the addition of tunicamycin on the basis of their mobility in SDS-polyacrylamide gels. As shown in Fig. 4, the transferrin receptor could still be labeled with [3H]palmitate in cells previously exposed to tunicamycin for as long as 48 h. Since the labeled receptor had a molecular weight of 95,000 corresponding to glycosylated molecules made prior to the addition of tunicamycin, it can be concluded that molecules that have been synthesized more than 48 h previously were labeled. The conclusion that transferrin receptors synthesized many hours earlier can still be labeled with palmitate was also supported by similar experiments in which protein synthesis was inhibited with emetine (10 μg/ml) as long as 48 h prior to labeling cells with palmitate (data not shown). Careful inspection of the autoradiographs shown in Fig. 4 also reveals that unglycosylated transferrin receptors were labeled with palmitate, suggesting that glycosylation is not obligatory for association of the receptors with fatty acid.

Fig. 4. Labeling of the transferrin receptor with [3H]palmitic acid in the presence of tunicamycin. Cells were incubated in the presence of tunicamycin (5 μg/ml) for 1.5 h and then labeled for 1.5 h with [3H]palmitic acid (Experiment 1), similar to conditions used for the results shown in Fig. 2. Alternatively, cells were incubated in the presence of tunicamycin (4 μg/ml) for 24, 36, and 48 h, then labeled for 4 h with [3H]palmitic acid (Experiment 2). The figure shows a fluorograph (18-day exposure for Experiment 1 and 22-day exposure for Experiment 2) of transferrin receptor immunoprecipitates prepared from cells incubated without tunicamycin (a and c) or with tunicamycin (b and d-f).

Fig. 5. Turnover rate of [35S]methionine ([35S-met])- or [3H]palmitate ([3H-palm])-labeled transferrin receptors. The figure shows autoradiograms of transferrin receptor (Tr) immunoprecipitates obtained after labeling CCRF-CEM cells with [35S]methionine (100 μCi/ml, 1 h) or [3H]palmitic acid (100 μCi/ml, 2 h), washing, then incubating in chase medium for 0, 12, 24, 36, 48, and 60 h as described under “Materials and Methods.” The autoradiograms were scanned and a semilog plot is shown for the percentage of integrated values (relative to the 0-h chase time point) versus hours of chase. [35S] Methionine-labeled transferrin receptors corresponding to 0-h chase had a faster migration on SDS-polyacrylamide gels than transferrin receptors for the remaining time points, since it corresponds to incomplete oligosaccharide-processed transferrin receptor molecules.

Fig. 3. Comparison of endo H digestion of the transferrin receptor labeled with [3H]palmitic acid or [35S]methionine. CCRF-CEM cells were pulse-labeled with [3H]palmitic acid for 10, 60, and 120 min without chasing (a-f) or pulse-labeled with [35S]methionine for 10 min, then chased for 0 or 240 min (g-j). Incubation in the absence of (-) or presence of endo H (+) was carried out on transferrin receptor immunoprecipitates as described under “Materials and Methods.” Exposure was for 19 days.
although judging from the amount of radioactivity associated with the unglycosylated receptor, tunicamycin may have a quantitative effect on the efficiency of the process.

**Discussion**

The results reported in this paper provide further information about the transferrin receptor of human cells. The mature cell surface glycoprotein contains both complex and high mannose N-asparagine-linked oligosaccharides, and all the evidence is consistent with these oligosaccharides being derived from a lipid-linked oligosaccharide precursor via a high mannose intermediate present on the immature intracellular form of the glycoprotein, as has been well established for other secreted and membrane-bound glycoproteins (23, 27, 31-34). The time required for oligosaccharide processing appears to be about 4 h, which is somewhat longer than that for the processing of the oligosaccharides attached to the vesicular stomatitis virus G glycoprotein (35). Inhibition of asparagine-linked glycosylation by tunicamycin does not completely block insertion of the glycoprotein into the plasma membrane. This is analogous to other membrane glycoproteins, including HLA (36) and IgM (37), but different from the Thy-1 glycoprotein from a mouse lymphoma mutant cell line where abnormal glycosylation completely prevented cell surface expression (38). As noted earlier, however, it is probable that tunicamycin treatment may well reduce the efficiency with which transferrin receptors reach the cell surface.

We have previously shown that the transferrin receptor can be labeled with [3H]palmitate and provided evidence that the fatty acid is probably covalently attached to the region of the molecule containing the membrane-associated portion of the molecule (19). Here we have shown that only the mature form of the receptor is associated with fatty acid and that transferrin receptors may be labeled with [3H]palmitate 48 h after their synthesis. These results differ in several respects from those reported for the attachment of fatty acids to the membrane glycoproteins of vesicular stomatitis and Sindbis viruses. The results reported by Schmidt and Schlesinger (39) suggest that fatty acid is bound to the viral glycoprotein shortly before oligosaccharide processing was complete, and they suggest the Golgi complex as a likely site for fatty acid incorporation into the glycoprotein. Further, nonglycosylated forms of the viral glycoprotein failed to act as fatty acid acceptors (40, 41), whereas we have detected [3H]palmitate labeling of the unglycosylated transferrin receptor found in tunicamycin-treated cells. The first difference may arise because, unlike the viral glycoproteins which are assembled into virions, transferrin receptors may, during their lifetime on the cell surface, be repeatedly internalized and recycled back to the plasma membrane, affording multiple opportunities for association with fatty acid, if, in fact, the Golgi complex, for example, is the site for incorporation. The fact that unglycosylated transferrin receptors act as fatty acid acceptors is because, unlike vesicular stomatitis virus G or Sindbis virus E1 and E2 glycoproteins, at least a fraction of the unglycosylated molecules reach the cell surface, and it is these molecules that are labeled with [3H]palmitate. Thus, as previously suggested (39-42), the failure of unglycosylated viral glycoproteins to reach the cellular site at which fatty acid addition occurs, rather than their structure per se, accounts for their inability to bind fatty acid.

The availability of monoclonal antibodies enabled us to investigate the turnover rate of transferrin receptors and the results clearly show that the protein moiety is degraded only slowly (t1/2 ~ 60 h). This means that, whatever the role of the receptor in iron transport, the transferrin receptor is not degraded in this process. Thus, if, as is widely believed, the receptor is internalized together with transferrin, it must be recycled back to the cell surface. The situation with the palmitate moiety of the receptor is less clear. The evidence suggests that fatty acid can be added to the protein moiety at any time after synthesis is completed and that it may be removed at a rate faster than can be accounted for by total degradation of the receptors. It has been calculated that the entire population of transferrin receptors may be internalized in about 6 min if this is obligatory for iron transport (7). If, then, addition and removal of fatty acid were a necessary event in this cycle, one would expect a turnover rate of [3H]palmitate molecules of the order of a few minutes. This does not appear to be the case.

**References**

1. Kaplan, J. (1981) Science 212, 14-20
2. Kahn, C. R. (1976) J. Cell Biol. 70, 281-286
3. Holley, R. W. (1980) J. Supramol. Struct. 13, 191-197
4. Hemmappardh, D., and Morgan, E. H. (1977) Br. J. Haematol. 35, 56-96
5. Sullivan, A. L., Grasso, J. A., and Weintraub, L. R. (1976) Blood 47, 133-143
6. Aisen, P., and Listowsky, I. (1980) Annu. Rev. Biochem. 49, 357-363
7. Karin, M., and Mintz, B. (1981) J. Biol. Chem. 256, 3245-3252
8. Morgan, E. H. (1981) Biochim. Biophys. Acta 642, 119-134
9. Jandl, J. H., and Katz, J. H. (1963) J. Clin. Invest. 42, 314-326
10. Seligman, P. A., Schleicher, R. B., and Allen, R. H. (1979) J. Biol. Chem. 254, 9943-9946
11. Hamilton, T. A., Wada, H. G., and Sussman, H. H. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 6406-6410
12. Larrick, J. W., and Cresswell, P. (1979) J. Supramol. Struct. 11, 579-586
13. Faulk, W. P., Hai, B., and Stevens, P. J. (1980) Lancet II, 390-392
14. Shindelman, J. E., Ortmeyer, A. E., and Sussman, H. H. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6406-6410
15. Omary, M. B., Trowbridge, I. S., and Minowada, J. (1980) Nature 286, 888-891
16. Trowbridge, I. S., and Omary, M. B. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3039-3043
17. Sutherland, R., Delia, D., Schneider, C., Newman, R., Kemhead, J., and Greaves, M. (1981) Proc. Natl. Acad. Sci. U. S. A., 78, 4615-4619
18. Goding, J. W., and Burns, G. F. (1981) J. Immunol., 127, 1256-1258
19. Omary, M. B., and Trowbridge, I. S. (1981) J. Biol. Chem. 256, 4715-4718
20. Minowada, J., Janossy, G., Greaves, M. F., Tsubota, T., Srivas-
Transferrin Receptor Biosynthesis

tava, B. I. S., Morikawa, S., and Tatsumi, E. (1978) J. Natl. Cancer Inst. 60, 1269–1276
21. Kessler, S. W. (1976) J. Immunol. 117, 1482–1489
22. Maizel, J. F. (1971) Methods Virol. 5, 179–246
23. Rothenberg, E., and Boyse, E. A. (1979) J. Exp. Med. 150, 777–791
24. Trowbridge, I. S., Ralph, P., and Bevan, M. J. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 157–161
25. Bonner, W. M., and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83–88
26. Tarentino, A. L., and Maley, F. (1974) J. Biol. Chem. 251, 811–816
27. Robbins, P. W., Hubbard, S. C., Turco, S. J., and Wirth, D. P. (1977) Cell 12, 893–900
28. Takatsuki, A., and Tamura, G. (1971) J. Antibiot. 24, 215–223
29. Takatsuki, A., Kohno, K., and Tamura, G. (1975) Agric. Biol. Chem. 39, 2089–2091
30. Octave, J.-N., Schneider, Y.-J., Crichton, R. R., and Trouet, A. (1981) Eur. J. Biochem. 115, 611–618
31. Li, E., Tabas, I., and Kornfeld, S. (1978) J. Biol. Chem. 253, 7762–7770
32. Kornfeld, S., Li, E., and Tabas, I. (1978) J. Biol. Chem. 253, 7771–7778
33. Owen, M. J., Kissonergis, A. M., and Lodish, H. F. (1980) J. Biol. Chem. 255, 9678–9684
34. Jokinen, M., Gahlberg, C. G., and Andersson, L. C. (1979) Nature 279, 604–607
35. Lodish, H. F., Braekev, W. A., Schwartz, A. L., Strous, G. J. A. M., and Zilberstein, A. (1981) Int. Rev. Cytol. Suppl. 12, 247–307
36. Ploegh, H. L., Orr, H. T., and Strominger, J. L. (1981) J. Immunol. 126, 270–275
37. Sibley, C. H., and Wagner, R. A. (1981) J. Immunol. 126, 1868–1873
38. Trowbridge, I. S., Hyman, R., and Mazauskas, C. (1978) Cell 14, 21–32
39. Schmidt, M. F. G., and Schlesinger, M. J. (1980) J. Biol. Chem. 255, 3334–3339
40. Schmidt, M. F. G., and Schlesinger, M. J. (1979) Cell 17, 813–819
41. Schmidt, M. F. G., Braekev, M., and Schlesinger, M. J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1687–1691
42. Zilberstein, A., Schneider, M., Paster, M., and Lodish, H. F. (1980) Cell 21, 417–427