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Use of a mutant cell line to study the kinetics and function of O-linked glycosylation of low density lipoprotein receptors

(Golgi complex/glycoprotein sorting/O-glycosylation/CHO cells/*LDLD* mutant line)

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ABSTRACT **A rapidly reversible defect in protein O-glycosylation exhibited by a line of mutant Chinese hamster ovary (CHO) cells was used to study the kinetics and function of O-glycosylation of the low density lipoprotein (LDL) receptor. The mutant line, genotype *LDLD*, cannot synthesize UDP-*N*-acetylgalactosamine under normal culture conditions and, therefore, cannot add mucin-type O-linked oligosaccharides to proteins. The UDP-*N*-acetylgalactosamine pools in *LDLD* cells can be filled rapidly when *N*-acetylgalactosamine is added to the culture medium, thus restoring normal synthesis of O-linked carbohydrates. Pulse–chase metabolic labeling experiments were used to show that (*i*) the first step in the O-glycosylation of LDL receptors can occur posttranslationally; (*ii*) after O-linked sugar-deficient LDL receptors reach the cell surface, they are not subject to subsequent O-linked sugar addition, suggesting that they do not return to compartments in which O-glycosylation takes place; (*iii*) O-linked carbohydrate chains on the LDL receptor itself are required for normal stability and function; and (*iv*) the instability of the O-linked sugar-deficient LDL receptor is due to proteolytic cleavage and the release into the medium of the bulk of the NH_2-terminal extracellular domain of the receptor. It appears that O-glycosylation of the LDL receptor and several other cell surface glycoproteins permits stable cell-surface expression by preventing proteolytic cleavage of the extracellular domains of these proteins.**

The low density lipoprotein (LDL) receptor is a glycoprotein that mediates the endocytosis of LDL, the principal cholesterol transporter in human plasma. The biosynthesis and structure of the LDL receptor have been described in detail (1). The human LDL receptor is synthesized as an approximately 120-kDa precursor form that contains high-mannose asparagine-linked (N-linked) oligosaccharide chains and incompletely formed serine/threonine-linked (O-linked) chains. Extensive processing in the rough endoplasmic reticulum (ER) and Golgi complex results in the conversion of the precursor to a 155- to 160-kDa mature form containing two complex N-linked glycans and approximately 18 mucin-like O-linked oligosaccharide chains. Typical structures of N-linked and O-linked oligosaccharides are shown in Fig. 1. The 35- to 40-kDa shift in apparent molecular mass of the receptor has been shown to be in large part due to the addition of the O-linked oligosaccharide chains. Approximately 70–85% of these oligosaccharides are clustered in an extracellular domain near the membrane-spanning domain of the receptor (and are designated the "clustered O-linked chains"), while the rest are apparently dispersed.

To study the structure, processing, and function of LDL receptors in cultured cells, we have developed methods to isolate mutant Chinese hamster ovary (CHO) cells with defects in LDL endocytosis (7–9). Somatic cell and molecular genetic methods have been used to identify four genes (*LDLA*, *LDLB*, *LDLC*, *LDLD*) required for LDL receptor function (10, 11). One of these, *LDLD*, is essential for the expression of UDPgalactose/UDP-*N*-acetylgalactosamine (GalNAc) 4-epimerase activity in CHO cells (12). The epimerase deficiency in *LDLD* cells leads to reversible (see below) defects in the synthesis of both N-linked and O-linked oligosaccharides on glycoproteins. The defect in the synthesis of all mucin-like O-linked chains directly or indirectly leads to the marked decrease in LDL receptor stability and function in *LDLD* cells.

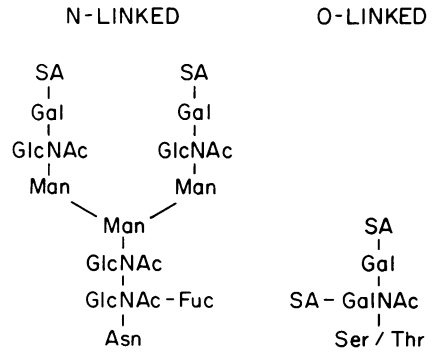

FIG. 1. Structures of typical N-linked and O-linked oligosaccharide chains. (*Left*) The complex N-linked chain structure shown is the major form of N-linked carbohydrate found on mature vesicular stomatitis virus G protein in CHO cells (2). The N-linked chains of the mature LDL receptor are also of the complex type (3, 4). (*Right*) The O-linked chain structure shown has been observed on the mature form of the LDL receptor in human A431 cells in culture (3). A similar structure has been found on LDL receptors synthesized in CHO cells (5). Note that GalNAc is the first sugar residue linked to the hydroxyl group of serine or threonine-linked mucin-like chains (6). SA, sialic acid; Ser/Thr, serine or threonine.

Davis *et al.* (5) have shown that the clustered O-linked sugar domain of the human LDL receptor can be removed by site-specific mutagenesis without adversely affecting the stability or function of receptors expressed in CHO cells of genotype *LDLA*. This finding raised the possibilities that the O-linked sugars required for receptor function in *LDLD* cells might be the dispersed sugars on the receptor itself or might be sugars that reside on proteins other than the LDL receptor. To test the latter possibility directly and to further examine the nature of O-glycosylation, we have exploited the rapid reversibility of the O-linked sugar defect in *LDLD* cells. The primary source of this defect is the inability of *LDLD* cells to add GalNAc to glycoproteins. GalNAc, the first sugar linked to protein during mucin-like O-linked chain synthesis (6), cannot be added because the epimerase deficiency blocks

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Abbreviations: LDL, low density lipoprotein; ER, endoplasmic reticulum; CHO, Chinese hamster ovary; O^d, O-linked deficient.

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synthesis of UDP-GalNAc, the substrate of the GalNAc transferase enzyme. Also, the addition of galactose to both O-linked and N-linked chains is blocked in *LDLD* cells because the same 4-epimerase deficiency prevents UDPgalactose synthesis. All of the phenotypic defects in *LDLD* cells can be reversed by adding GalNAc and galactose to the culture medium (12). These exogenous sugars can be internalized and converted to UDP-GalNAc and UDPgalactose by salvage pathways that bypass the epimerase defect. In the current work, we have found that the O-linked sugars on the receptor itself are essential for maintaining normal receptor stability and function. The O-linked deficient receptors, some of which can be detected on the cell surface, are rapidly cleaved near the membrane-spanning domain, and much of their NH_2-terminal extracellular domain is released into the medium. Furthermore, these studies show that O-glycosylation can occur posttranslationally and that the rapid reversibility of the glycosylation defects in *LDLD* cells may be useful for studying the processing and intracellular sorting of other glycoproteins.

MATERIALS AND METHODS

Materials and Cell Culture. Materials were obtained and cell culture at 37°C was performed as described (4, 12). *LDLD* cells transfected with an expression vector for the Epstein–Barr virus major antigen envelope glycoprotein (K.K., M. Silberklang, and M.K., unpublished data) were used for the experiment shown in Fig. 5.

Immunoprecipitation Procedures. On day 0, *LDLD* cells were seeded into six-well dishes (150,000 cells per well) in 3 ml of medium A [Ham's F-12 medium supplemented with 100 units of penicillin and 100 μg of streptomycin per ml, 2 mM glutamine, and 3% (vol/vol) newborn calf lipoprotein-deficient serum] containing additional galactose (20 μM) and GalNAc (400 μM) as indicated. On day 2, the cell monolayer in each dish was washed twice in methionine-free Ham's F-12 medium and then incubated in methionine-free medium A supplemented with [^{35}S]methionine for the indicated times. The cells were washed with complete Ham's F-12 medium and chased in medium A supplemented with 1 mM unlabeled methionine. Labeling and chase media contained the same supplements of exogenous sugars as the initial growth medium. Unless otherwise noted, the amount of GalNAc added (400 μM) was optimized for the rapid and complete correction of LDL receptor structure; we previously have shown that less GalNAc (100 μM) is adequate when cells are grown in its presence for 2 days.

After the chase, the cell monolayers and chase media were independently collected, solubilized with detergents, and subjected to immunoprecipitation with anti-LDL receptor antibodies, anti-C or anti-R, and to electrophoresis and autoradiography as described (4). Intact cells were removed from the medium by centrifugation for 5 min at 4°C and 2000 rpm in a Beckman TJ-6R centrifuge prior to addition of detergents and processing (4). Treatment of immunoprecipitates with endoglycosidase H was performed as described (4).

RESULTS

The distinctive properties of *LDLD* cells have permitted the examination of the effects of O-glycosylation of the LDL receptor on receptor stability and function. We have shown (12) that the low intracellular pools of UDP-GalNAc and UDPgalactose in *LDLD* cells can be filled rapidly by adding GalNAc and galactose to the culture medium. This raised the possibility of manipulating culture and metabolic labeling conditions so that a mixed population of normally glycosylated and O-linked deficient (O^d) [^{35}S]methionine-labeled receptors could be expressed and monitored in the same cells simultaneously. If the two different structural forms of the receptor were degraded at the same rate under these conditions, some trans-acting cellular component must be determining receptor stability. On the other hand, if the O^d receptors exhibited their characteristic instability while their normally glycosylated neighbors exhibited normal stability, one could conclude that the O-linked chains on the receptor itself were critical for establishing receptor stability.

The experiment shown in Fig. 2 was performed to determine if it were possible to simultaneously label normally glycosylated and O^d receptors. Unless otherwise noted, in this and the other experiments shown below, 20 μM galactose was included in all media to insure the synthesis of normal N-linked chains under all conditions. This galactose supplement also permitted the synthesis of complete O-linked chains when GalNAc was present. In the experiment shown in Fig. 2 *Left*, cells were pulse-labeled with [^{35}S]methionine for 5 min and chased for a total of 90 min with medium containing unlabeled methionine. At the indicated times of chase, 400 μM GalNAc was added to the chase medium. Cells were harvested and subjected to immunoprecipitation with an anti-LDL receptor antibody, and the precipitates were analyzed by gel electrophoresis and autoradiography.

Most of the labeled LDL receptors could be O-glycosylated normally (processed to the 155-kDa mature form, "m"; ref. 4), even when GalNAc was added as late as 15 min after the pulse. Thus, normal O-linked glycosylation can occur posttranslationally. Adding GalNAc 1 hr after the pulse was not sufficient to permit any detectable normal O-glycosylation. Instead, all of the receptor was in the O^d form. At intermediate times of addition, cells contained both the normal mature and O^d forms of the receptor, rather than receptors of intermediate size. We conclude that there is a critical period when newly synthesized receptors reside in or proximal to compartments in which the first step of O-glycosylation can be performed or are in the appropriate conformations to permit this first step of O-linked glycosylation. At increasing times after this critical period, increasing

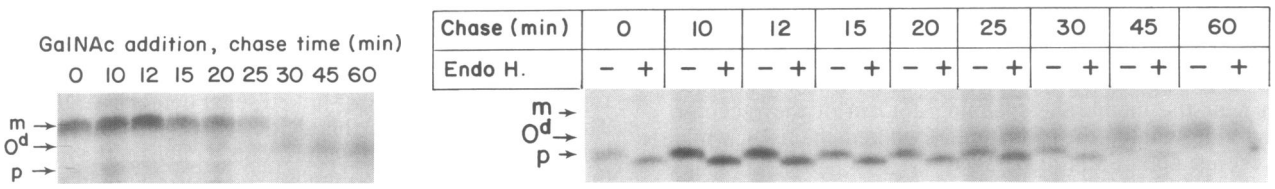

FIG. 2. Effects of varying the time of GalNAc addition on O-linked glycosylation of the LDL receptor. (*Left*) *LDLD* cells were plated and grown in medium A supplemented with 20 μM galactose, pulse-labeled for 5 min with 600 μCi (1 Ci = 37 GBq) of [^{35}S]methionine per ml, and chased for 90 min. At the indicated times of chase, 400 μM GalNAc was added. After the chase, the cells were harvested and solubilized, and the mixture was subjected to immunoprecipitation, electrophoresis, and autoradiography as described. m, 155-kDa mature form of the LDL receptor; p, 125-kDa precursor form. The O^d form was 130–145 kDa. (*Right*) In the same experiment, *LDLD* cells were grown, pulse-labeled as described above, chased in medium containing galactose without GalNAc, and harvested at the indicated times of chase. The cell extracts were subjected to immunoprecipitation, treatment with or without endoglycosidase H (endo H), electrophoresis, and autoradiography as described.

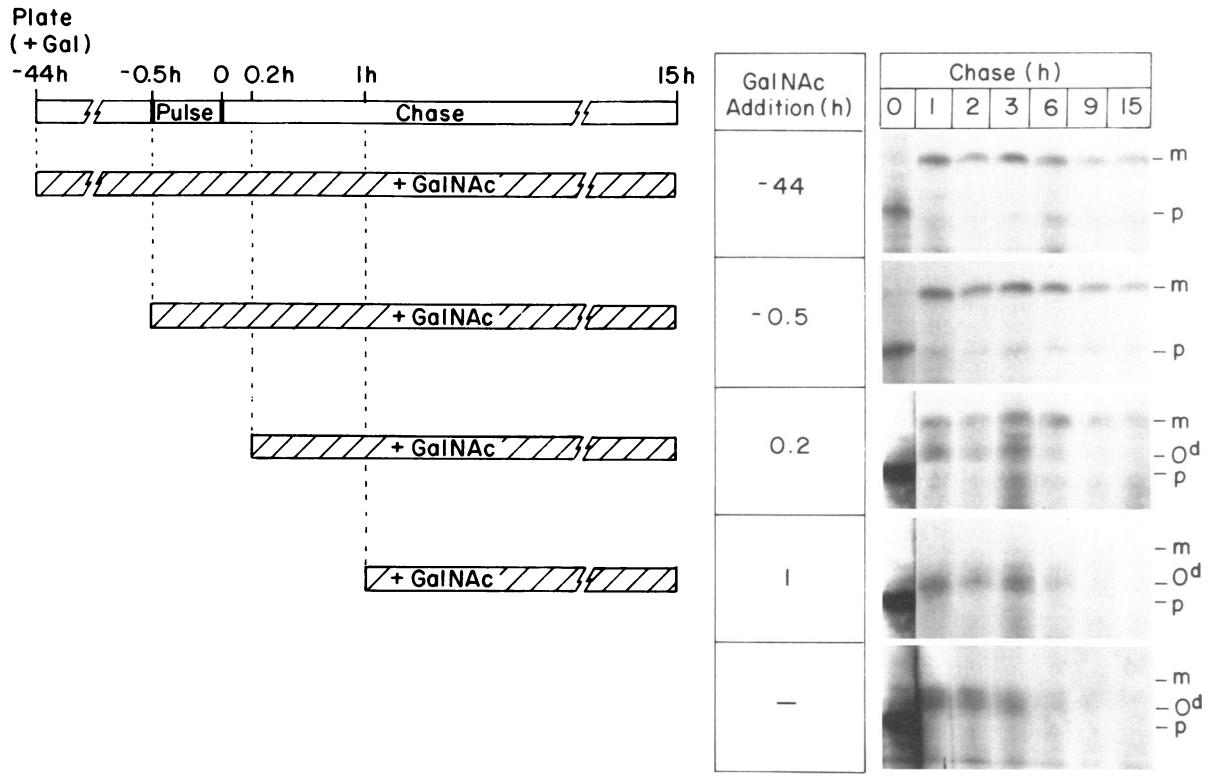

FIG. 3. Effects of varying the time of GalNAc addition on LDL receptor stability. *LDLD* cells were plated and grown in medium A supplemented with 20 μM galactose, pulse-labeled for 30 min with 400 μCi of [^{35}S]methionine per ml, and chased for the indicated times. GalNAc was added at the time of plating (−44 hr), at the time of pulse (−0.5 hr), at 0.2 hr of chase, or at 1 hr of chase; or no GalNAc was added (−). The cells were harvested, solubilized, and subjected to immunoprecipitation, electrophoresis, and autoradiography as described. GalNAc was added at 0.2 hr of chase rather than 0.5 hr as in Fig. 2 because of the longer pulse period.

amounts of receptor move beyond these compartments and are no longer susceptible to GalNAc addition. LDL receptors become resistant to the effects of GalNAc addition (Fig. 2 *Left*) at essentially the same time that their N-linked chains become resistant to endoglycosidase H (Fig. 2 *Right*). This time corresponds to the time at which the dramatic shift from the precursor to the mature forms takes place (see *Discussion*). Although exogenous GalNAc had a profound effect on the O-glycosylation of the receptors, it had no effect on the rate at which LDL receptors became resistant to endoglycosidase H (data not shown), suggesting that GalNAc addition does not affect the time course of receptor processing.

The experiment described above established the feasibility of following the fate of normal and O^d receptors in the same cells simultaneously. Fig. 3 shows a set of pulse–chase experiments in which the stabilities of normal and O^d LDL receptors were measured. The relative amounts of normal and O^d forms of the receptor were varied by changing the time of GalNAc addition. Time 0 corresponds to the beginning of the chase period, which immediately followed a 0.5-hr pulse labeling. Virtually all of the receptor was chased into the stable mature form when GalNAc was added at −44 hr or −0.5 hr (Fig. 3, top two panels). Only the unstable O^d form was synthesized when GalNAc was never added or when addition was withheld until 1 hr into the chase (Fig. 3, bottom two panels). When GalNAc was added at 1 hr of chase, the O^d form was never subsequently converted to the mature form despite the continued presence of GalNAc during the remainder of the chase. When GalNAc was added at 0.2 hr of chase (Fig. 3, middle panel), the population of receptors was processed into both forms. A cell-surface Pronase sensitivity assay (4, 13) was used to show that both forms of the receptor were displayed on the cell surface at chase times of 1 hr or longer (Fig. 4 and data not shown).

The heterogeneity of the receptor population observed when GalNAc was added at 0.2 hr of chase could have arisen either because there was one homogeneous population of cells, all of which contained two forms of the receptor, or because there were two distinct populations of cells, each bearing different forms of the receptor. The latter case appears highly unlikely because the relatively long (30 min) pulse-labeling period used in this experiment should have insured an adequately heterogeneous population of receptors in each individual cell (compare with the rate of receptor processing seen in Fig. 2). We conclude that in the experiment in Fig. 3, the cell population was most probably homogeneous and that most of the cells contained two different forms of the receptor. Therefore, under these conditions in which both forms of the receptor were expressed on the same cell surfaces simultaneously, the normal

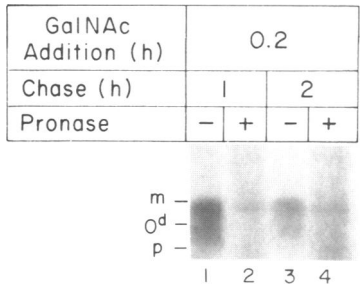

FIG. 4. Pronase sensitivity of LDL receptors in *LDLD* cells. *LDLD* cells were grown and labeled as described in Fig. 3. At 0.2 hr of chase, GalNAc was added. At the indicated times of chase, the monolayers were incubated for 20 min in the absence or presence of Pronase (20 μg/ml at 37°C) and then harvested as described (4, 13). Cell extracts were subjected to immunoprecipitation, electrophoresis, and autoradiography as described.

mature form exhibited normal stability and the O^d form exhibited its characteristic instability. Thus, the O-linked chains that are crucial for receptor stability reside on the receptor protein itself (see below).

To begin to examine the mechanism responsible for receptor instability, we performed the pulse–chase experiment shown in Fig. 5. Cells were grown, metabolically labeled and chased in medium supplemented with the indicated sugars, and solubilized for subsequent immunoprecipitation procedures with either anti-C, an anti-peptide antibody that recognizes the COOH-terminal cytoplasmic domain of the receptor, or anti-R, which recognizes the extracellular NH_2-terminal domain (4). Cell extracts were subjected to immunoprecipitation with anti-C (Fig. 5 *Left*); and the media, with anti-R (Fig. 5 *Right*) and anti-C (not shown). As reported (12), newly synthesized, cell-associated receptors were very unstable in the absence of GalNAc (Fig. 5, upper two panels). The rapid loss of cell-associated receptors was accompanied by the coincident appearance of receptor in the medium. In the presence of GalNAc (Fig. 5, lower two panels), cell-associated receptors were relatively stable, and there was much less receptor detected in the medium. Under all conditions, the LDL receptors in the medium that were precipitated by anti-R could not be detected with the anti-C antibody. These results suggest that the marked instability of cell-associated O^d LDL receptors is a consequence of the proteolytic cleavage of the O^d receptor and the consequent release of a large N-terminal fragment into the medium. Similar, but much less extensive, proteolysis/release of receptors with truncated (GalNAc alone) or normal (both sugars) O-linked chains also occurred.

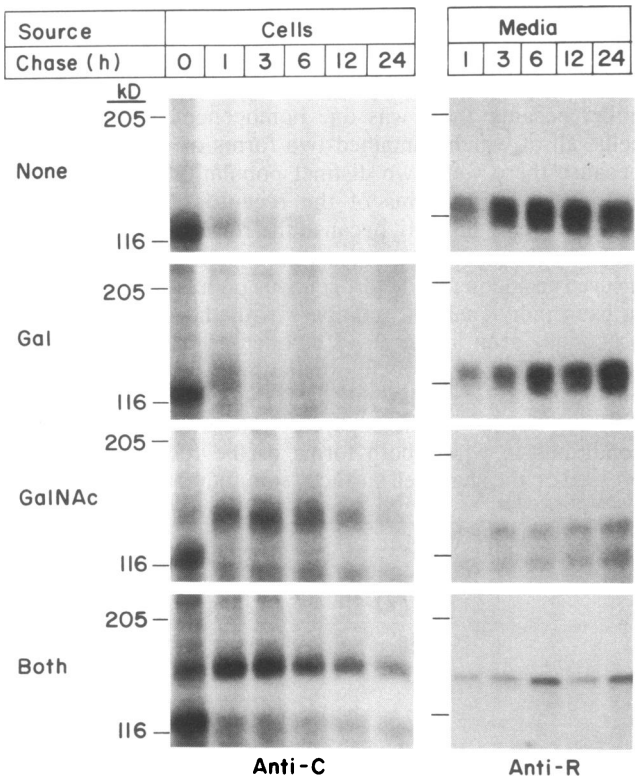

FIG. 5. Effects of sugar additions on the release of LDL receptors into the culture media. *LDLD* cells were plated, grown, pulse-labeled for 30 min with 450 μCi of [^{35}S]methionine per ml, and chased in unlabeled medium for the indicated times, all in media containing the indicated additions of galactose (20 μM) and/or GalNAc (200 μM) as described. Comparable amounts of cells and media were collected and subjected to immunoprecipitation procedures, electrophoresis, and autoradiography as described.

DISCUSSION

The UDPgalactose/UDP-GalNAc 4-epimerase deficiency of the CHO cell *LDLD* mutant causes a fully reversible defect in mucin-like O-linked glycosylation of proteins such as the LDL receptor (12). In the absence of exogenous sources of GalNAc, the *LDLD* cells cannot synthesize UDP-GalNAc and, thus, cannot add the first sugar of mucin-like O-linked chains to the serine and threonine side chains of glycoproteins. This defect can be corrected by adding GalNAc to the culture medium, and, thus, represents a powerful tool that complements the use of inhibitors of N-linked glycosylation, such as tunicamycin (14), for the study of glycoprotein synthesis, structure, and function.

We have previously shown that the availability of GalNAc plays a critical role in establishing LDL receptor stability and function in CHO cells (12). In the current work, we have exploited the rapidly reversible O-glycosylation defect of *LDLD* cells to determine if O-linked oligosaccharides residing on the LDL receptor itself are essential for receptor stability. In these experiments, *LDLD* cells were grown and metabolically labeled by a protocol that permitted the simultaneous examination of the stabilities of normally glycosylated and O^d LDL receptors in a single population of cells. Newly synthesized, normally glycosylated LDL receptors exhibited normal stability, while newly synthesized, O^d receptors simultaneously showed their characteristic instability. Thus, it appears that O-linked glycosylation of the receptor itself, rather than glycosylation of some other cellular component, is responsible for the O-linked sugar-dependence of LDL receptor stability and function.

The critical O-linked chains on the receptor have not yet been identified. The receptor contains clustered and dispersed O-linked chains (1), either or both of which might contribute to receptor stability. Deletion of the clustered O-linked domain of the human LDL receptor does not alter its stability or function in CHO cells exhibiting normal glycosylation (5). Nevertheless, initial studies of the mechanism underlying the instability of cell-associated O^d receptors suggest that the clustered O-linked sugars may play a critical role. These experiments showed that O^d receptor instability was due to proteolytic cleavage and the release into the medium of the bulk of the N-terminal extracellular portion of the receptor. The large proteolytic fragment was recognized by antibodies specific for the NH_2 terminus of the receptor but could not be detected by antibodies specific for the COOH-terminal domain. Because most of the apparent mass of the receptor was released into the medium, it seems likely that proteolysis occurred on the extracellular portion of the receptor near the membrane-spanning domain, probably within or near the clustered O-linked domain. Access to the protease-sensitive site(s) is apparently blocked by normal O-glycosylation. Experiments comparing the stability and function of full-length and clustered O-linked domain-deleted human LDL receptors in *LDLD* cells should help to clarify the role of the clustered O-linked sugars in protecting the receptor from this proteolysis.

In a series of related studies (unpublished data), we found that the stable, cell-surface expression of three other, rather diverse, O-glycosylated membrane proteins in *LDLD* cells was also dependent on GalNAc. In the absence of GalNAc addition, we observed dramatic reductions in the surface expression of the human interleukin 2 receptor (α chain; K.K., S. Call, S. Dower, and M.K.), decay-accelerating factor (P. Reddy, I. Caras, and M.K.), and the major antigen envelope protein of Epstein–Barr virus (EBVenv; K.K., M. Silberklang, and M.K.). As with the O^d LDL receptor, both O^d decay-accelerating factor and O^d EBVenv could be detected on the cell surface and were much less stable than their normally glycosylated counterparts, and large extracel-

lular fragments were released into the media. In contrast, O^d interleukin 2 receptors exhibited essentially normal stability but were missorted so that they were not expressed on the cell surface. These findings suggest that O-glycosylation of cell-surface proteins may frequently play an important role in determining their cell-surface expression, often by controlling protein stability. Additional studies will be required to identify the cellular compartment(s) in which proteolysis occurs and to characterize the proteolytic activity. Results with normally glycosylated LDL receptors in wild-type (unpublished data) and *LDLD* cells suggest that proteolysis/release may be involved in the normal degradation of LDL receptors.

The rapid reversibility of the glycosylation defect in *LDLD* cells has also allowed us to investigate the kinetics of initial synthesis of O-linked carbohydrate chains on LDL receptors. Previous studies of the timing of the initial events in O-glycosylation have given a variety of results. While some studies suggest that GalNAc addition occurs in the ER (15, 16), others show that GalNAc addition probably occurs in the Golgi (17–26) or may occur in either compartment (27). The current studies have shown that GalNAc addition may occur posttranslationally. Normal O-linked glycosylation of LDL receptors took place even when GalNAc was provided to the cells 15 min after metabolic labeling. Within the time resolution of these experiments, loss of receptor sensitivity to GalNAc addition (Fig. 2) was coincident with the modification of N-linked carbohydrate chains in the medial Golgi (development of endoglycosidase H resistance; ref. 28). These data are consistent with, but do not prove, models proposing that GalNAc addition in CHO cells takes place in late or transitional ER or in early Golgi stacks. Further experiments with *LDLD* cells used in conjunction with cell fractionation and ultrastructural techniques may help to resolve this question.

Experiments by Snider and Rogers (29, 30) show that a mammalian cell-surface glycoprotein can be recycled to Golgi compartments after initial synthesis, processing, and transport to the cell surface. Their experiments involved the enzymatic removal of sugars from glycoproteins on the cell surface and the subsequent analysis of the replacement of those sugars by cellular Golgi-associated glycosyl transferases. The reversibility of the glycosylation defects in *LDLD* cells provides an alternative method for applying their strategy to studying membrane protein recycling. In place of enzymatically removing sugars from surface-labeled glycoproteins, *LDLD* cells may be used to synthesize pulse-labeled glycoproteins without galactose and/or GalNAc. Once the abnormally glycosylated proteins are expressed at the cell surface or in a particular cellular organelle, these sugars can be added to the culture medium, repopulating the sugar-nucleotide pools. The rates and extents of subsequent ER/Golgi-associated sugar addition can then be determined. This approach can be used only when the abnormal glycosylation of a protein does not prevent its initial delivery to the appropriate cellular compartment (in the case of the LDL receptor, the cell surface). In the experiment shown in Fig. 3 in which chase times extended to 15 hr, we found that O^d LDL receptors on the cell surface never underwent further glycosylation. These results suggest that O^d LDL receptors, which are abnormally unstable, either cannot recycle to cellular compartments in which they can be O-glycosylated or are proteolytically cleaved before such recycling can occur. An alternative possibility is that because of the absence of O-linked carbohydrates, the O^d receptors folded into conformations that prevented any further O-glycosylation. Additional GalNAc and galactose addback experiments with the LDL receptor and other glycoproteins should be useful for evaluating the general role of the ER–Golgi complex in the sorting and recycling of glycoproteins.

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1. Goldstein, J. L., Brown, M. S., Anderson, R. G. W., Russell, D. W. & Schneider, W. J. (1985) *Annu. Rev. Cell Biol.* **1**, 1–39.
2. Stanley, P. (1985) in *Molecular Cell Genetics: The Chinese Hamster Cell*, ed. Gottesman, M. M. (Wiley, New York), pp. 745–772.
3. Cummings, R. D., Kornfeld, S., Schneider, W. J., Hobgood, K. K., Tolleshaug, H., Brown, M. S. & Goldstein, J. L. (1983) *J. Biol. Chem.* **258**, 15261–15273.
4. Kozarsky, K. F., Brush, H. A. & Krieger, M. (1986) *J. Cell Biol.* **102**, 1567–1575.
5. Davis, C. G., Elhammer, A., Russell, D. W., Schneider, W. J., Kornfeld, S., Brown, M. S. & Goldstein, J. L. (1986) *J. Biol. Chem.* **261**, 2828–2838.
6. Kornfeld, R. & Kornfeld, S. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans*, ed. Lennarz, W. J. (Plenum, New York), pp. 1–32.
7. Krieger, M., Brown, M. S. & Goldstein, J. L. (1981) *J. Mol. Biol.* **150**, 167–184.
8. Krieger, M., Martin, J., Segal, M. & Kingsley, D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5607–5611.
9. Krieger, M., Kingsley, D. M., Sege, R. D., Hobbie, L. & Kozarsky, K. F. (1985) *Trends Biochem. Sci.* **10**, 447–452.
10. Krieger, M. (1983) *Cell* **33**, 413–422.
11. Kingsley, D. M. & Krieger, M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5454–5458.
12. Kingsley, D. M., Kozarsky, K. F., Hobbie, L. & Krieger, M. (1986) *Cell* **44**, 749–759.
13. Tolleshaug, H., Hobgood, K. K., Brown, M. S. & Goldstein, J. L. (1983) *Cell* **32**, 941–951.
14. Struck, D. & Lennarz, W. J. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans*, ed. Lennarz, W. J. (Plenum, New York), pp. 35–83.
15. Strous, G. J. A. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2694–2698.
16. Patzelt, C. & Weber, B. (1986) *EMBO J.* **5**, 2103–2108.
17. Abeijon, C. & Hirschberg, C. B. (1987) *J. Biol. Chem.* **262**, 4153–4159.
18. Elhammer, A. & Kornfeld, S. (1984) *J. Cell Biol.* **99**, 327–331.
19. White, D. A. & Speake, B. K. (1980) *Biochem. J.* **192**, 297–301.
20. Hanover, J. A., Elting, J., Mintz, G. R. & Lennarz, W. J. (1982) *J. Biol. Chem.* **257**, 10172–10177.
21. Kimura, J. H., Lohmander, L. S. & Hascall, V. C. (1984) *J. Cell. Biochem.* **26**, 261–278.
22. Lohmander, L. S., Hascall, V. C., Yanagishita, M., Kuettner, K. E. & Kimura, J. H. (1986) *Arch. Biochem. Biophys.* **250**, 211–227.
23. Hanover, J. A., Lennarz, W. J. & Young, J. D. (1980) *J. Biol. Chem.* **255**, 6713–6716.
24. Niemann, H., Boschek, B., Evans, D., Rosing, M., Tamura, T. & Klenk, H.-D. (1982) *EMBO J.* **1**, 1499–1504.
25. Shida, H. & Matsumoto, S. (1983) *Cell* **33**, 423–434.
26. Roth, J. (1984) *J. Cell Biol.* **98**, 399–406.
27. Spielman, J., Rockley, N. L. & Carraway, K. L. (1987) *J. Biol. Chem.* **262**, 269–275.
28. Dunphy, W. G. & Rothman, J. E. (1985) *Cell* **40**, 463–472.
29. Snider, M. D. & Rogers, O. C. (1985) *J. Cell Biol.* **100**, 826–834.
30. Snider, M. D. & Rogers, O. C. (1986) *J. Cell Biol.* **103**, 265–275.