II. Biosynthesis and Turnover of a \( M_r = 110,000 \) Glycoprotein Localized to the Hepatocyte Bile Canaliculus

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Antiserum was raised in rabbits against a bile canalicular glycoprotein of \( M_r = 110,000 \) purified to homogeneity from rat liver. The antiserum specifically immunoprecipitated a \( M_r = 110,000 \) polypeptide from hepatocytes metabolically labeled with \(^{[35]S}\)methionine. When hepatocytes in primary culture were incubated with tunicamycin before labeling with \(^{[35]S}\)methionine in the presence of tunicamycin, the major polypeptide immunoprecipitated by the specific antiserum from Triton X-100 extracts of cells had a molecular weight of 59,000. Enzymatic removal of \( N \)-linked carbohydrates from the \( M_r = 110,000 \) glycoprotein by \( N \)-glycanase digestion also yielded a polypeptide with a molecular weight of 85,000. In pulse-chase experiments using \(^{[35]S}\)methionine, the \( M_r = 110,000 \) protein detected by the specific antiserum first appears as \( M_r = 85,000 \) and \( 75,000 \) intermediate species, which are endoglycosidase H sensitive. The \( M_r = 85,000 \) intermediate form is lost first with time followed by the \( M_r = 75,000 \) form giving rise to the \( M_r = 110,000 \) form that is endoglycosidase H resistant. Neuraminidase digestion of the \( M_r = 110,000 \) form generated an \( M_r = 85,000 \) form but with a different carbohydrate structure than the intermediate \( M_r = 85,000 \) form detected in the pulse-chase experiments. The time required to accomplish the processing of the \( M_r = 85,000 \) and \( 75,000 \) forms is relatively slow. Finally, the terminal sugars are added and the mature \( M_r = 110,000 \) glycoprotein is rapidly transported to the cell surface. A minimum time of 90 min is required for the \( M_r = 110,000 \) bile canalicular glycoprotein to be synthesized, processed, and reach the cell surface which is long relative to the time required (10 min) for another domain-specific protein, the receptor for asialoglycoproteins, to reach the sinusoidal surface. The \( M_r = 110,000 \) bile canalicular glycoprotein turns over in the bile canalicular domain with a half-life of 43 h while the asialoglycoprotein receptor turns over in the sinusoidal domain with a half-life of 23 h.

Hepatocytes in the liver are polarized cells having a highly differentiated and complex plasma membrane. This membrane contains at least the following morphologically and functionally distinct regions or domains: a sinusoidal domain containing coated pits which is involved in secretion and uptake of a variety of small and large molecular weight material from the hepatic portal blood supply; a bile canalicular domain which is involved in the secretion of bile salts synthesized by the hepatocyte and transport of bile salts recaptured by the hepatocyte from the serum at the sinusoidal domain; gap junctions which are involved in the transport of ions and other small molecules between adjacent hepatocytes; and, a basal lateral domain which is involved in the secretion of the constituents forming the basal lamina and in attaching the hepatocyte to the basal lamina. Separating these different domains of the plasma membrane are tight junctions. Each of these domains of the plasma membrane contains a characteristic set of proteins which allow the domain to perform its differentiated function. The sinusoidal domain contains proteins that are involved, for example, in the uptake of a variety of hormones and macromolecules including asialoglycoproteins from the serum. The bile canalicular domain contains proteins that are involved in the transport of bile salts, albeit such proteins have not yet been unequivocally identified. How hepatocytes and other polarized cells establish and maintain the characteristic domains of their plasma membrane is an important area of investigation and is the subject of this paper. We have in previous studies identified three glycoprotein antigens that are constituents of the bile canalicular membrane fraction of liver denoted by us as \( N_2a \) (1). In the accompanying manuscript (2) we have purified and characterized a \( M_r = 110,000 \) glycoprotein from this membrane fraction. A monospecific polyclonal antiserum was prepared in rabbits against the purified protein and the \( M_r = 110,000 \) glycoprotein was localized by immunocytochemical techniques to the bile canaliculus. As described in this manuscript this antiserum was used in combination with metabolic labeling in combination with other methods to examine the pathway of biogenesis of the \( M_r = 110,000 \) glycoprotein in primary cultures of rat hepatocytes. The pathway of biogenesis of this glycoprotein is complex and includes the accumulation within the cell of two relatively long-lived intermediates of \( M_r = 85,000 \) and \( 75,000 \). The latter intermediate then is converted into the \( M_r = 110,000 \) mature form of the glycoprotein which is transported relatively rapidly to the plasma membrane. The time required to accomplish the synthesis, intracellular processing, and the delivery of the \( M_r = 110,000 \) bile canalicular domain-specific glycoprotein to the plasma membrane is much longer than the time required by the hepatocyte for the biogenesis and delivery to the membrane of a sinusoidal domain-specific glycoprotein, the receptor for galactose-terminated glycoproteins (asialoglycoproteins). The \( M_r = 110,000 \) bile canalicular glycoprotein is also more stable with respect to its turnover behavior than is the asialoglycoprotein receptor; the receptor turns over in the plasma membrane with a half-life of about 23 h while the \( M_r = 110,000 \) bile canalicular glycoprotein has a 43-h half-life.

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**EXPERIMENTAL PROCEDURES**

**Materials**—Na<sup>140</sup> and [<sup>35</sup>S]methionine (800 Ci/mmole) were purchased from Amersham Corp. and Dulbecco’s Modified Eagle Medium. Protein A-Sepharose 4B was obtained from Pharmacia Fine Chemicals. Triton X-100 was purchased from Accurate Chemical (Westbury, NY). Endoglycosidase H was purchased from Miles Laboratories (Liale, IL), and N-glycanase was obtained from Genzyme (Boston, MA). Neuraminidase was bought from Sigma. Buffalor rais were obtained from M. A. Bioproducts (Walkersville, MD). DME^- and fetal calf serum were from Gibco. Collagen was purchased from the Collagen Corp. (Palo Alto, CA).

**Preparation of Antibodies**—The preparation of antisera against GP 110 and the asialoglycoprotein receptor was previously described (2, 3). IgG was prepared from serum through a series of NH<sub>4</sub>SO<sub>4</sub> precipitations. Unless otherwise noted the IgG fraction was used in all experiments.

**Isolation of Hepatocytes**—Hepatocytes were isolated from perfused Buffalo rat liver essentially as described by Petell and Doyle (4). Cells were cultured on collagen-coated tissue culture dishes or flasks. Normal 1-2 x 10<sup>6</sup> cells were plated per 35-mm dish or 25-cm<sup>2</sup> T-flask. This was a working ratio for scaling up or down in the cell number plated in different experiments, see figure legends for changes. For metabolic labeling experiments, cells were cultured for at least 2 h to allow them to attach to the dish. The monolayers were washed gently with DMEM, 10% fetal calf serum to remove cells that were not attached prior to biosynthetic labeling.

**Surface Iodination of Hepatocytes**—Surface iodination of hepatocytes was carried out as previously described (4).

**Metabolic Labeling of Cultured Hepatocytes**—Hepatocytes were cultured for at least a 2-h time period after isolation as described above. They were then incubated in DMEM minus methionine for 60 min. The DMEM lacking methionine was removed and fresh DMEM minus methionine was added followed by [<sup>35</sup>S]methionine (800 Ci/mmole). See figure legends for amounts of labeled amino acid used, length of labeling, and chase with unlabeled methionine, if any.

**Immuno precipitation of GP 110**—Samples for immunoprecipitation were prepared at 4 °C in INPATS (2% Triton X-100, 0.5% SDS, 50 mM sodium phosphate buffer, pH 7.4, 0.15 M NaCl, and 1 mM phenylmethylsulfonyl fluoride). Samples were either homogenized or sonicated and the extracts were then centrifuged 100,000 × g, 30 min. Bovine serum albumin was added to the supernatant to a final concentration of 1%. Incubation with antibodies was allowed to proceed overnight at 4 °C or for 2 h at room temperature. Thereafter excess protein A-Sepharose was added and incubation was continued for 1 h either at 4 °C overnight or at room temperature for 2 h. The immune precipitates were isolated by repeated washes with INPATS containing 1% bovine serum albumin. The antigens were released from immune precipitates of iodinated material by boiling the samples in Sds sample buffer, whereas [<sup>35</sup>S]methionine-labeled proteins were eluted by incubating the immune complexes in 0.2 M glycine, pH 2.5, for 30 min. Supernatant fractions obtained via either method were analyzed by SDS-PAGE followed by fluorography. As shown in Fig. 1, pre-immune IgG recognized no radioactive polypeptides while anti-GP 110 immunoprecipitated radioactive polypeptides were analyzed by SDS-PAGE followed by fluorography. As shown in Fig. 1, pre-immune IgG recognized no radioactive polypeptides while anti-GP 110 immunoprecipitated radioactive polypeptides were analyzed by SDS-PAGE followed by fluorography.

**Turnover Studies**—After cells were iodinated they were washed with DMEM, 10% fetal calf serum and were cultured in the same medium. Following the appropriate incubation, 0–72 h, cultures were rinsed with phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride, scraped with a rubber policeman, and centrifuged to collect the cells. Aliquots were removed for DNA determinations and cell number was normalized prior to immunoprecipitation.

**DNA Determination**—DNA content per sample was determined as described by Labarca and Paigen (5).

**Electrophoresis of Samples**—Two-dimensional gel analysis was performed according to O’Farrell (6). Single dimensional gels were run as described by Laemmli (7).

**RESULTS**

**Identification of Intermediates in the Synthesis of GP 110**—In the preceding paper (2) we reported the purification and preparation of monospecific rabbit antisera against an M<sub>r</sub> = 110,000 glycoprotein, denoted GP 110. GP 110 was shown by immunocytochemical and cell biological studies to be predominantly localized in the bile canalculus of rat hepatocytes. Biochemical characterization of GP 110 revealed that GP 110 has an approximate pl of 4.9 and was extensively glycosylated containing approximately eight N-linked complex carbohydrate chains. Upon removal of N-linked carbohydrates by N-glycanase treatment the apparent molecular weight of GP 110 as judged by two-dimensional PAGE could be reduced to M<sub>r</sub> = 59,000 with a pl of 5.3.

To gain more insight into the cellular mechanisms for the biogenesis of GP 110, rat hepatocytes were cultured in the presence or absence of tunicamycin followed by metabolic labeling with [<sup>35</sup>S]methionine. In tunicamycin minus experiments, rat hepatocytes were continuously labeled with [<sup>35</sup>S]methionine for 5 h. Detergent extracts of labeled cells were incubated with pre-immune or anti-GP 110 rabbit IgG and subsequently incubated with Protein A-Sepharose beads. The immunoprecipitated radioactive polypeptides were analyzed by SDS-PAGE followed by fluorography. As shown in Fig. 1, pre-immune IgG recognized no radioactive polypeptides while anti-GP 110 immunoprecipitated a M<sub>r</sub> = 110,000 polypeptide synthesized by rat hepatocytes. In addition, two other polypeptides were detected with M<sub>r</sub> of 75,000 and 85,000. Proteolytic digest experiments suggested that the two smaller immunoprecipitated polypeptides were related to GP 110. Thus, the smaller sized polypeptides appear to be intermediates in the biosynthesis of GP 110.

**Fig. 1.** Effect of tunicamycin on the biosynthesis of the M<sub>r</sub> = 110,000 bile canalicular glycoprotein. Hepatocytes were metabolically labeled with [<sup>35</sup>S]methionine for 5 h and extracts were prepared and analyzed as described under "Experimental Procedures." Lane 1, extract incubated with pre-immune serum; lane 2, extract incubated with anti-GP 110; lane 3, hepatocytes were treated with tunicamycin, 5 μg/ml, prior to and during metabolic labeling as above and immunoprecipitated with anti-GP 110. All samples were analyzed by SDS-PAGE.
As tunicamycin is known to block the dolichol-dependent N-linked glycosylation of proteins, a radioactive precursor product that does not contain N-linked carbohydrate moieties should accumulate in methionine-labeled hepatocytes. Cultured primary hepatocytes were incubated with tunicamycin for 5 h followed by labeling with \(^{35}S\)methionine for 5 h also in the presence of tunicamycin. In immunoprecipitates, the majority of radioactivity was associated with a \(M_r = 59,000\) polypeptide (Fig. 1). However, under the experimental conditions employed tunicamycin did not entirely block dolichol transfer as some GP 110 and its synthesis intermediates were also present. These results corroborate previous experiments showing that the apparent molecular weight of GP 110 without N-linked carbohydrates is 59,000. Hence, GP 110 is initially synthesized as a polypeptide precursor with an apparent \(M_r = 59,000\) which is eventually converted to a glycoprotein of \(M_r = 110,000\).

**Biosynthesis of GP 110 in Cultured Rat Hepatocytes**—To elucidate the relationship of mature GP 110 and its two intermediate forms of \(M_r = 75,000\) and 85,000 the biosynthesis of GP 110 was studied in pulse-chase type experiments with radioactive methionine. Cultured hepatocytes were exposed for 30 min to \(^{35}S\)methionine and subsequently incubated in medium containing unlabeled methionine. For each point in the pulse-chase experiment detergent extracts of labeled hepatocytes were prepared and immunoprecipitated with anti-GP 110 IgG. As shown in Fig. 2A, after the initial 30-min labeling period only two radioactive polypeptides were recovered by immunoprecipitation; a predominant \(M_r = 75,000\) polypeptide and a smaller amount of the \(M_r = 85,000\) form. In the subsequent chase period, a minor amount of mature GP 110 is observed together with the \(M_r = 75,000\) and \(85,000\) forms. Eventually the \(M_r = 85,000\) form of the protein disappears leaving only the \(M_r = 75,000\) intermediate and the more abundant \(M_r = 110,000\) M, polypeptide. After 6 h, all of the synthesis intermediates have been converted to mature GP 110. In comparison, we also examined the biosynthesis of the asialoglycoprotein receptor (ASGR) in similar pulse-chase experiments (Fig. 2B). After the initial 10-min pulse all of the radioactivity immunoprecipitated by antibodies against ASGR was found associated with the mature form of ASGR. In fact, we can only detect synthesis intermediates of ASGR when cells are treated with tunicamycin prior to labeling (data not shown).

To assess more accurately the time required for newly synthesized GP 110 to reach the cell surface of the hepatocyte. Hepatocytes were incubated at 37 °C in medium containing \(^{35}S\)methionine for pulse followed by incubation in medium containing unlabeled methionine for chase for a specified length of time as just described. At different times of the pulse-chase experiments hepatocytes in situ were incubated with either antibodies against GP 110 or ASGR at 4 °C. As seen in Fig. 3A, newly synthesized GP 110 is first detected at the cell surface at approximately 90 min of chase. As expected, only the \(M_r = 110,000\) form of GP 110 is present at the cell surface. In parallel experiments, the time required for asialoglycoprotein receptor to appear at the cell surface was also investigated employing antibodies against ASGR. It was found that the mature form of ASGR was detected at the cell surface in a substantially shorter period of time, approximately 10 min (Fig. 3B).

**Processing of the Carbohydrate Structure of GP 110**—The carbohydrate structures of GP 110 and the two 75,000 and 85,000 synthesis intermediates were investigated by examining their sensitivity to digestion with enzymes reactive with

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**FIG. 2. Kinetics of biosynthesis of the \(M_r = 110,000\) bile canalicular glycoprotein relative to the sinusoidal receptor for asialoglycoproteins.** A, 1-2x10⁶ hepatocytes were pulsed with \(^{35}S\)methionine (1 μCi/ml) for 30 min and then chased with medium containing unlabeled methionine for the times indicated. Extracts were prepared from cells as described under "Experimental Procedures" and were incubated with anti-GP 110. Samples were analyzed by SDS-PAGE. Molecular weights are indicated.

**FIG. 3. Kinetics of appearance at the cell surface of the \(M_r = 110,000\) bile canalicular glycoprotein relative to the receptor for asialoglycoproteins.** Hepatocytes were labeled as described in the legend to Fig. 2. After labeling, cells were washed with serum-free DMEM, brought to 4 °C, and incubated with the appropriate antibodies at 4 °C while gently shaking. After a 90-min incubation unreacted antibodies were removed by rinsing cells with ice-cold phosphate-buffered saline. Cells were removed from dishes with a rubber policeman and processed as described under "Experimental Procedures."
high mannose and hybrid-type carbohydrate chains or all N-linked carbohydrate moieties, endoglycosidase H or N-glycanase, respectively. In the first experiment, hepatocytes were labeled at the cell surface via lactoperoxidase-catalyzed iodicination and the cell extracts were reacted with antibodies against GP 110. It was observed that mature GP 110 at the cell surface was sensitive to N-glycanase (Fig. 4, lanes A and B) while insensitive to endoglycosidase H (data not shown). In companion experiments, cells were labeled metabolically with [35S]methionine and chased with unlabeled methionine to ensure that GP 110 and its two intermediate forms were labeled at the cell surface via lactoperoxidase-catalyzed iodicination (Fig. 4, lane C). Immunoprecipitated material from detergent cell extracts were then treated with these hydrolyses (Fig. 4, lanes D and E). N-Glycanase effectively deglycosylated N-linked chains from all GP 110 biosynthetic forms while only the synthesis intermediates were sensitive to the endoglycosidase H. These results indicate that the M_r = 75,000 and 85,000 forms contain either high mannose or hybrid-type carbohydrate structures and are probably restricted to the endoplasmic reticulum/early Golgi compartments of the hepatocytes.

To rule out the possibility that the M_r = 85,000 synthesis intermediate described here and the M_r = 85,000 form of GP 110 produced by neuraminidase treatment did not possess the same carbohydrate structures, immunoprecipitated GP 110 products were treated with endoglycosidase H and/or neuraminidase. Hepatocytes were labeled at the cell surface via lactoperoxidase-catalyzed iodicination. The treatment of cell surface GP 110 with endoglycosidase H has no effect on the membrane protein; only synthetic intermediates in the biosynthetic intermediate and the neuraminidase-treated cell surface GP 110 do not possess the same carbohydrate structures even though they have the same apparent molecular weight. Thus, these two forms represent two distinct steps in the processing of carbohydrate moieties of GP 110 which probably occurs in the endoplasmic reticulum and the trans region of the Golgi.

**Turnover of GP 110**—The turnover of hepatocyte plasma membrane proteins proceeds at heterogeneous rates. The time required for one-half of the GP 110 to turn over in the hepatocyte was determined in a classical manner. Freshly isolated hepatocytes were labeled via lactoperoxidase-catalyzed iodicination at 4 °C to preferentially label plasma membrane proteins which contain externally exposed tyrosine residues. It was previously shown that GP 110 on the cell surface is readily accessible to this type of labeling methodology (see Ref. 2). Iodinated hepatocytes were used to seed tissue culture plates for duplicate time points. Then, hepatocytes were cultured at 37 °C until the cells were harvested and equivalent number of cells for each time point were extracted in the presence of detergent. Extracts representing individual time points were incubated with excess amounts of antibody to GP 110. The immunoprecipitated products were subjected to SDS-PAGE and visualized by autoradiography. The radioactive bands corresponding to GP 110 were cut out and counted. As depicted in Fig. 6, the t_1/2 or half-life, of GP 110 was determined to be approximately 43 h. As a control, the half-life of asialoglycoprotein receptor was measured concomitantly. In agreement with past studies, the t_1/2 of asialoglycoprotein receptor was found to be about 23 h.

**Fig. 4. Effect of N-glycanase and endoglycosidase H on the M_r = 110,000 bile canalicular glycoprotein and its metabolic intermediates.** Immunopurified GP 110 was treated with the enzymes as indicated in the figure. Lanes A and B, GP 110 was immunoprecipitated from extracts prepared from iodinated hepatocytes. Lanes C–E, hepatocytes were metabolically labeled by pulse-chase methods with [35S]methionine in such a manner so that there were approximately equal amounts of M_r = 75,000 and 85,000 form and mature M_r = 110,000 forms of the bile canalicular domain-specific protein.

**Fig. 5. Effect of neuraminidase and endoglycosidase H on the M_r = 110,000 glycoprotein and its intermediates.** Immunopurified GP 110 was treated with enzymes as indicated in the figure. Lanes A–D, GP 110 was immunoprecipitated from extracts prepared from iodinated hepatocytes.
washed, and then cultured for 0-72 h. At the times indicated cells were harvested. Cell number was normalized and processed as described in the text.

Glycoprotein and the receptor for asialoglycoproteins and hepatocytes in culture. These proteins have an apparent Mr of 110,000 and is localized in the bile canalculus. In fact it is one of the more abundant proteins in this membrane domain and is readily purified by lectin affinity chromatography and preparative polyacrylamide gel electrophoresis. This behavior is typical of membrane glycoproteins and is due primarily to heterogeneity in the number and composition of the carbohydrate chains on the single polypeptide backbone of the glycoprotein (8, 9). The purified preparation of the Mr = 110,000 glycoprotein elicited in rabbits an immunological response producing a monospecific polyclonal antibody of sufficiently high titer to perform localization and biogenetic studies.

Studies using tunicamycin, an inhibitor of N-linked glycosylation, show that the polypeptide backbone of the Mr = 110,000 glycoprotein has an Mr of about 59,000 as assessed by PAGE. Treatment of the Mr = 110,000 glycoprotein with endoglycosidase H, an enzyme capable of removing all N-linked carbohydrate structures from mature glycoproteins produces a set of fragments with the smallest also having an Mr, also of 59,000 as assessed by PAGE. intermediates in the conversion of the Mr = 59,000 polypeptide to the mature Mr = 110,000 glycoprotein residing in the bile canalculus were identified by both pulse-chase labeling experiments with [35S]methionine as metabolic precursor and with inhibitors of glycosylation and enzymes that act on carbohydrate substrates of known composition in glycoproteins. As a result of these studies we propose the following scheme for the pathway of biogenesis of the Mr = 110,000 bile canalicular membrane glycoprotein in hepatocytes (Fig. 7). To the growing polypeptide chain of ultimate Mr = 59,000 in the rough endoplasmic reticulum are added as many as eight N-linked glycans of the high mannose-type which are subsequently processed in the endoplasmic reticulum to form the Mr = 85,000 precursor which is endoglycosidase H sensitive. The Mr = 85,000 precursor is further processed in the endoplasmic reticulum or in early cis stacks of the Golgi into the Mr = 75,000 precursor which is still endoglycosidase H sensitive. The fact that there exists a significant pool of both the Mr = 85,000 and 75,000 intermediates suggests "pause" points in the biogenesis of this plasma membrane glycoprotein. One pause point may be at the junction of the endoplasmic reticulum/Golgi regions of the cell. As the Mr = 75,000 form of the maturing glycoprotein traverses through the cis, medial, and trans regions of the Golgi it is further processed to yield a complex carbohydrate structure which is endoglycosidase H resistant. The mature glycoprotein of 110,000 is then transported relatively rapidly from the trans region of the Golgi to the plasma membrane, since no Mr = 110,000 mature form of the glycoprotein can be detected in intracellular membrane compartments of the hepatocyte. Treatment of the Mr = 110,000 mature form of the bile canalicular domain-specific glycoprotein with neuraminidase converts it in vitro to a Mr = 85,000 endoglycosidase-resistant species which may represent a late step in the biogenesis of this glycoprotein in situ which we have not yet been able to identify in the metabolic-labeling studies. It

**DISCUSSION**

In this article we have initiated a study of the pathway of biogenesis of a plasma membrane domain-specific glycoprotein of rat hepatocytes. This glycoprotein has an apparent Mr of 110,000 and is localized in the bile canalculus. In fact it is one of the more abundant proteins in this membrane domain and is readily purified by lectin affinity chromatography and preparative polyacrylamide gel electrophoresis. After purification the glycoprotein migrated as a single somewhat charge and size heterogeneous species when subjected to two-dimensional polyacrylamide gel electrophoresis. This behavior is typical of membrane glycoproteins and is due primarily to heterogeneity in the number and composition of the carbohydrate chains on the single polypeptide backbone of the glycoprotein (8, 9). The purified preparation of the Mr = 110,000 glycoprotein elicited in rabbits an immunological response producing a monospecific polyclonal antibody of sufficiently high titer to perform localization and biogenetic studies.

Studies using tunicamycin, an inhibitor of N-linked glyco-
requires at least 90 min for the mature $M_t = 110,000$ species of the glycoprotein to reach the plasma membrane of the hepatocyte where it can react with its specific antibody. In contrast, newly synthesized mature rat receptor for asialoglycoproteins, also known as the rat hepatic lectin for galactose-terminated glycoproteins, can traverse the endomembrane system of the hepatocyte and be accessible for reaction with its specific antibody at the cell surface in about 10 min in the same isolated cultures of hepatocytes. The asialoglycoprotein receptor is localized exclusively in the sinusoidal domain of the hepatocyte.

The rat receptor for asialoglycoproteins, in addition to being present in a different plasma membrane domain than the $M_t = 110,000$ glycoprotein, is also smaller in molecular weight and has a fewer number of carbohydrate side chains associated with it. The rat receptor consists of three distinct molecular weight forms of 43,000, 50,000, and 59,000, which are encoded by two or three distinct gene products in rats (10). The size of the polycode backbone of each of the three forms is about 30,000 with carbohydrate modifications accounting for the additional molecular weight of the three forms. No intermediates in the biogenesis of this rat hepatic lectin can be detected unless tunicamycin is used to inhibit N-linked glycosylation. The site where the asialoglycoprotein receptor and the $M_t = 110,000$ bile canicular protein enter the plasma membrane is not yet known. Furthermore, the molecular/cellular mechanisms responsible for the differential delivery and the time required to effect delivery of these two glycoproteins to their different domains of residence in the plasma membrane of the hepatocyte are not yet known but are being examined by us and others (11–20). The hepatocyte in culture is in fact an experimentally manipulatable model system to study the way in which a highly polarized cell establishes its complex and different plasma membrane domains. However, better methods of cell fractionation, which we are attempting to develop for hepatocytes in culture (21), are needed before studies can be done to answer these questions concerning intracellular transport of plasma membrane proteins in polarized cells.

The metabolic turnover of the two domain-specific proteins also differs; the asialoglycoprotein receptor turns over in the sinusoidal domain of hepatocytes with a half-life of about 1 day while the $M_t = 110,000$ glycoprotein of the bile canicular domain turns over with a 43-h half-life, similar to the half-life for turnover of dipeptidyl peptidase another bile canicular domain-specific protein in hepatocytes (22). The asialoglycoprotein receptor functions in the receptor-mediated endocytosis of galactose-terminated glycoproteins and is continually cycling between the plasma membrane and intracellular compartments (3, 23–28). In fact, there is a relatively large amount of the receptor present in intracellular membranes of the hepatocyte at all times (21). In contrast, there is very little of the mature $M_t = 110,000$ bile canicular glycoprotein present in intracellular membrane compartments of the hepatocyte and it is doubtful that this membrane protein cycles at all. The difference in turnover rate between the two domain-specific plasma membrane proteins may in fact be due to differences in their ability to cycle between the plasma membrane and intracellular membrane compartments. As part of the cycling pathway the asialoglycoprotein receptor may have more frequent access to the lysosomal compartment and its degradative system than does the $M_t = 110,000$ bile canicular glycoprotein. Despite considerable effort by several groups of investigators in recent years the mechanism used by the hepatocyte to degrade proteins present in the plasma membrane is still not known with certainty (3, 29–38). This cell most likely uses the lysosomal system to degrade interiorized units of plasma membrane. But, superimposed upon bulk degradation of membrane constituents in lysosomes there is degradation of some membrane proteins by nonlysosomal hydrolases most likely present inside the cell, in the membrane itself, and in the milieu exterior to the cell. Which of these systems is responsible for the differential turnover behavior of the sinusoidal and bile canicular membrane proteins described herein also remains to be elucidated.

REFERENCES


2 A. V. Le and D. Doyle, unpublished observations.