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The two subunits of the human asialoglycoprotein receptor have different fates when expressed alone in fibroblasts

(galactose lectin/subunit assembly/receptor biosynthesis)

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ABSTRACT **Two related polypeptides, H1 and H2, comprise the human asialoglycoprotein receptor (ASGP-R). Stable lines of murine NIH 3T3 fibroblasts expressing H1 alone or H2 alone do not bind or internalize the ligand asialoorosomucoid (ASOR), which contains triantennary oligosaccharides. In contrast, cells expressing H1 and H2 together bind and degrade ASOR with properties indistinguishable from those of the ASPG-R in human hepatoma HepG2 cells. Whether or not H2 is coexpressed, H1 is synthesized as a 40-kDa precursor bearing high-mannose oligosaccharides, processed to its mature 46-kDa form, and transported to the cell surface. In cells expressing only H1, homodimers and -trimers of H1 are formed. In contrast, when expressed in 3T3 cells without H1, H2 is synthesized as its 43-kDa precursor, bearing high-mannose oligosaccharides, but is rapidly degraded. When H1 and H2 are coexpressed in the same cell, the H1 polypeptide "rescues" the H2 polypeptide; H2 is processed to its characteristic 50-kDa mature form and is transported to the surface. We conclude that the human ASGP-R is a multichain heterooligomer, probably a trimer of H1 molecules in noncovalent association with one, two, or three H2 molecules, and that the two polypeptides normally interact early in biosynthesis.**

The hepatocyte asialoglycoprotein receptor (ASGP-R) binds with high-affinity serum glycoproteins bearing multiple terminal galactose or *N*-acetylgalactosamine residues. It has served as a model for many of the events of receptor-mediated endocytosis, such as ligand binding, receptor–ligand dissociation and sorting, and recycling of the receptor to the cell surface (1–3). The human ASGP-R, purified from liver or the hepatoma cell line HepG2, was thought to be composed only of a single 46-kDa polypeptide, designated H1. However, by cDNA cloning (4, 5) and direct identification (6), a second and less abundant human ASGP-R molecular species, H2 (49–50 kDa), has been characterized. Are these two human ASGP-R polypeptides independent receptors with different functions and specificities, or do they only together comprise a functional ASGP-R?

McPhaul and Berg (7) demonstrated that expression of both rat ASGP-R cDNAs, rat hepatic lectin (RHL)-1 and RHL-2/-3, is required to form a functional ASGP-R in rat HTC cells, one that can internalize and accumulate in lysosomes fluorescent-labeled asialoorosomucoid (ASOR). Sawyer *et al.* (8) showed that plasma membrane RHL-1 and RHL-2/-3 peptides do interact noncovalently. In contrast, on the basis of chemical cross-linking and galactose affinity chromatography studies, Drickamer and his colleagues concluded that the rat ASGP-R polypeptides associate only into distinct homooligomers of RHL-1 or RHL-2/-3 (9). In particular, they could not chemically cross-link RHL-1 with RHL-2/-3, but they were able to cross-link each respective peptide to itself to form homooligomers (9).

Using chemical cross-linking and antibody-induced degradation protocols, we showed that, in HepG2 cells, H1 and H2 form a heterooligomer (10). Here we examine the fate of the individual polypeptides in singly transfected mouse NIH 3T3 cells or in 3T3 cells that express both human ASGP-R polypeptides. We propose a model of the ASGP-R structure in which a "core" trimer of H1 molecules can interact with one, two, or three H2 molecules. Although the exact stoichiometry of the interactions between H1 and H2 is still unknown, it is clear that these polypeptides must interact early in biosynthesis to assemble a functional ASGP-R.

MATERIALS AND METHODS

Construction of pDOL-ASGP-R. All DNA manipulations involved standard techniques (11). The retroviral expression vector pDO-L was kindly provided by A. Korman, C. Cepko, and R. C. Mulligan of the Whitehead Institute (12). The entire coding regions of H1 and H2 were excised from the plasmids pSA1 and pSA2 (4, 5) and ligated into the *Bam*HI site of pDO-L via *Bgl* II and *Bam*HI linkers, respectively.

Introduction of ASGP-R Polypeptides into NIH 3T3 Cells. Ten micrograms of pDOL-H1, pDOL-H2, or pDOL without insert DNA was used to transform ψ-2 cells (12, 13) as described by Deitcher *et al.* (14). The transient viral supernatant was used to infect NIH 3T3 cells, which were selected for G418 resistance and cloned as described (12, 14). Cell lines engineered to contain both ASGP-R polypeptides were made by reinfecting a parental cell line with the appropriate transient viral supernatant followed by dilution cloning. Clones were screened for the presence of the H2 or H1 cDNA by differential hybridization, using the cytoplasmic dot blot procedure of White and Bancroft (15) and the hybridization conditions described by Spiess and Lodish (5).

Ligand Binding, Uptake, and Degradation Assay. Human orosomucoid (Sigma) was desialylated as described except that Hepes-buffered saline (HBS) (50 mM Hepes/141 mM NaCl/3.8 mM KCl, pH adjusted to 7.41 with NaOH) was used instead of phosphate-buffered solution (16). ASOR was iodinated with Enzymobeads (Bio-Rad) according to the manufacturer's protocol. The ligand binding uptake and degradation assay used is similar to that described by Schwartz *et al.* (17, 18) except HBS was used in place of phosphate-buffered saline.

Metabolic Labeling. Confluent or near confluent (75–100%) cells in 60-mm tissue culture plates were labeled with [^{35}S]cysteine as described (6, 10).

Immunoadsorption. Monolayers of metabolically labeled cells were washed twice in ice-cold HBS and then lysed with

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Abbreviations: ASGP-R, asialoglycoprotein receptor; ASOR, asialoorosomucoid; ^{125}I-ASOR, ^{125}I-labeled ASOR; RHL, rat hepatic lectin; DFDNB, difluorodinitrobenzene.

800 μl of a solution of 1% Triton X-100/0.5% sodium deoxycholate/2 mM phenylmethylsulfonyl fluoride in HBS (lysis buffer) at 4°C. The suspension was transferred to a microcentrifuge tube, mixed in a Vortex for 60 sec, kept 1 hr at 4°C, and then centrifuged for 20 min at 15,000 × *g*. The supernatant was used for immunoadsorptions and for protein and radioactivity determination.

Typically 100 μg of total cell protein was diluted to a total vol of 400 μl of lysis buffer. Samples were incubated with normal rabbit serum (used at 1:400) for at least 1 hr at 4°C, followed by addition of 35 μl of a 50% suspension of protein A agarose (BRL). After incubation for another hour at 4°C, the sample was centrifuged for 5 min at 15,000 × *g*. To the supernatant was added the anti-H1 antibody (H1-COOH) at 1:40 dilution or the anti-H2 antibody (cytoplasmic domain) at 1:50 dilution (6, 10); it was incubated overnight at 4°C or for 3 hr at room temperature. Seventy-five microliters of protein A agarose was then added and the tubes were incubated for at least 1 more hr. We washed the protein A agarose five times with a solution containing 0.5% SDS, 1% Triton X-100, 0.5 deoxycholate, 1% bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride in HBS, and twice with HBS. The antibody–antigen complexes were eluted from the protein A agarose by boiling for 5 min in 100 μl of SDS/polyacrylamide gel sample buffer, containing dithiothreitol (19). The supernatant was then subjected to SDS/PAGE on 10% polyacrylamide gel (19) and fluorography (20). Autoradiographs were scanned with an LKB laser microdensitometer.

Chemical Cross-Linking. Solutions of difluorodinitrobenzene (DFDNB) in absolute ethanol were made fresh for each experiment. Total microsomal membranes were isolated as described (9, 10). Cross-linking was carried out for 1 hr at room temperature as described (10) except reactions were quenched in SDS/polyacrylamide gel sample buffer.

Immunoblot with Anti-H1 Antibody. The samples were subjected to electrophoresis on 7.5% SDS/polyacrylamide gels and then transferred to nitrocellulose paper. The membrane was incubated with a 1:500 dilution of anti-H1 antibody and then with ^{125}I-labeled protein A (^{125}I-protein A) following an established protocol (21). ^{125}I-protein A-labeled bands were visualized by autoradiography.

RESULTS

Both H1 and H2 Polypeptides Are Required for Normal ASGP-R Function. We generated a number of stable cell lines expressing H1 alone or H2 alone as well as several mock-infected cell lines that contained only the pDOL vector, and we performed standard ligand binding assays with ^{125}I-labeled ASOR (^{125}I-ASOR) on at least 15 clones from each group. Ligand binding by cells expressing H1 or H2 alone was indistinguishable from that obtained with control 3T3 cells or mock-transfected cells (data not shown). This low level of binding was unaffected by a 200-fold excess of unlabeled ASOR and was thus deemed to be nonspecific. HepG2 cells, a positive control, routinely bound ≈10 times more ^{125}I-ASOR, and >90% of this was bound specifically.

To test the hypothesis that both polypeptides are required to form a functional receptor, we generated two cell lines (1-7-1 and 1-7-2) that express both H1 and H2. Both cell lines exhibited significant specific binding of the ligand ASOR: 274 and 80 pg of ASOR per mg, as compared with 132 pg of ASOR per mg in HepG2 cells. The difference in extent of ligand binding by the 1-7-1 and 1-7-2 cells may be due to differences in expression of the H2 polypeptide, since both were derived from the same H-1-expressing 1-7 cells. Fig. 1 documents that 1-7-1 cells bind ^{125}I-ASOR specifically. Addition of 200 μg of unlabeled ASOR per ml reduced binding of ^{125}I-ASOR to the same level as in mock-infected or singly infected cells, indicating that >90% of the ligand is bound specifically.

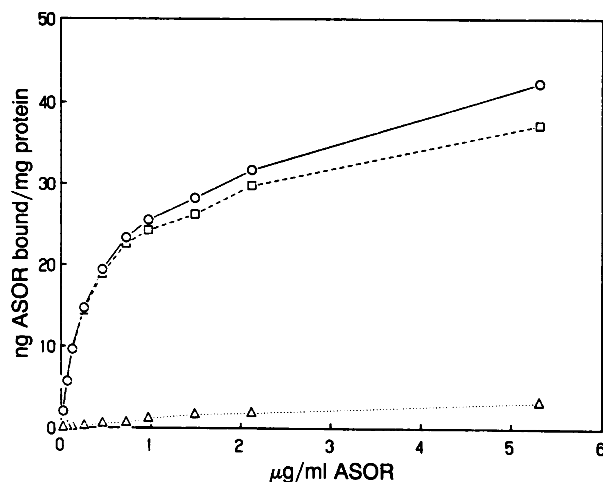

FIG. 1. Saturation binding analysis. Cells were incubated with various concentrations of ^{125}I-ASOR (2×10^6 cpm/μg) at 4°C for 2 hr, with (△) or without (○) an excess (200 μg/ml) of nonradioactive ASOR, these representing the nonspecific and total binding, respectively. □, Difference between total and nonspecific binding, deemed to be specific binding. All points are the means of triplicate determinations.

When the data from Fig. 1 were plotted according to the Scatchard equation, a single class of high-affinity binding sites with an apparent K_d of 6.5 mM for ASOR is observed (not shown), which is similar to the K_d value of 7.5 nM for the ASGP-R in HepG2 cells (17). The number of ASGP-R molecules on the surface of 1-7-1 cells is 0.78 pmol per mg of protein.

As a further measure of receptor function, we examined the internalization and degradation of ^{125}I-ASOR in 1-7-1 cells during a continuous incubation at 37°C (Fig. 2). The amount of cell-associated radioactivity reached a plateau between 1 and 2 hr; ^{125}I degradation products appeared in the medium by 1 hr and increased at a linear rate thereafter. The sum of these two values represents the cumulative amount of cell-associated ^{125}I-ASOR: 0.056 pmol of ASOR per min per mg of protein for hours 1.5–5. From these values, we can calculate a cycle time for the ASGP-R: the time required for ligand binding to the receptor, internalization of the receptor–

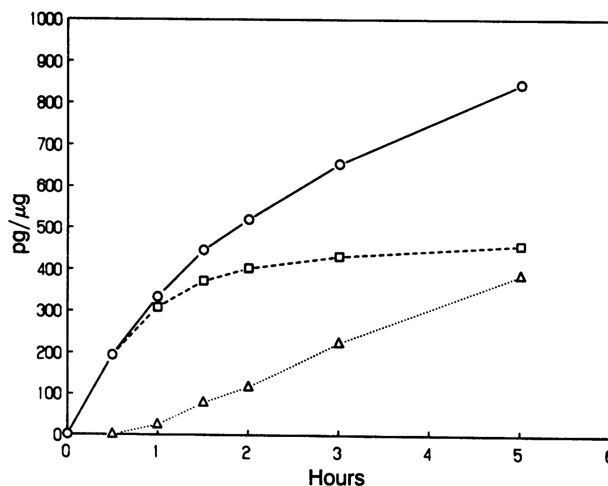

FIG. 2. Uptake and degradation of ^{125}I-ASOR in 1-7-1 cells at 37°C. Tissue culture dishes (60 mm) of 1-7-1 cells were washed and incubated with ^{125}I-ASOR (2 μg/ml) for various times at 37°C. At each time, a portion of the medium was removed and analyzed for ^{125}I degradation products. The cells were rinsed and analyzed for cell-associated radioactivity. □, Total cell-associated radioactivity; △, ^{125}I degradation products in the medium; ○, sum of the two values. All points represent the mean of triplicate determinations.

ligand complex, dissociation of ligand, and return of the receptor to the surface. This time equals the total receptor number divided by the cumulative rate of ligand uptake (17). Since 50% of the total receptor population in the cell is on the surface (below), the total number of receptors in 1-7-1 cells is 1.56 pmol per mg of protein and the cycle time for the ASGP-R in 1-7-1 cells is calculated to be ≈28 min (1.56 pmol·mg^{-1} per 0.056 pmol·min^{-1}·mg^{-1}). This is similar to the value determined for HepG2 cells (17).

Expression of the H1 ASGP-R Polypeptide in NIH 3T3 Cells. We studied the synthesis, processing, and localization of the ASGP-R polypeptides in representative cell lines expressing either H1 or H2 or both together. Fig. 3*A* shows that H1 polypeptide expressed alone (1-7 cells) or coexpressed with the H2 polypeptide (1-7-1 cells) is synthesized and processed similarly to H1 in HepG2 cells. In all three cell lines, a predominant 40-kDa H1 immunoreactive polypeptide was labeled during the 20-min pulse (hour 0). In 1-7 and 1-7-1 cells (at hour 0), this species is sensitive to endoglycosidase H as it is in HepG2 cells (data not shown) and presumably is in the endoplasmic reticulum or other pre-Golgi compartment. A minor 34-kDa band is likely to be nonglycosylated H1, which disappears during the chase period. In 1-7 cells during the chase period (lanes 3–6 from the left) the 40-kDa H1 precursor disappears; ≈50% is converted into the 46-kDa mature form and the rest is degraded. As in HepG2 cells, this 46-kDa species is resistant to endoglycosidase H (not shown) and thus has been transported at least to the medial Golgi. HepG2 cells under the same conditions exhibit a faster and slightly more efficient (≈70%) processing of the precursor to the mature 46-kDa species. The 1-7-1 cells exhibit a pattern of H1 synthesis and processing identical to HepG2 cells.

Expression of the H2 ASGP-R Polypeptide in Transfected 3T3 Cells. Similar experiments performed on cell lines expressing only H2, such as 2-18, yielded quite different results. After a 20-min pulse, 2-18, 1-7-1, and HepG2 cells all contained a single radiolabeled 43-kDa H2 precursor band (Fig. 3*B*, 0 hr). In all cells, this H2 precursor is sensitive to endoglycosidase H (data not shown), suggesting that it is glycosylated and is localized to the endoplasmic reticulum. In 1-7-1 and HepG2 cells, the 43-kDa precursor is processed to the mature 50-kDa H2 species at a rate similar to that observed for H1 in these cells (compare to Fig. 3*A*). However, in the 2-18 cell line, which expresses H2 alone, a 50-kDa mature form of the receptor is not observed at any time during the pulse or chase. Instead, the 43-kDa protein is degraded rapidly with an apparent half-life of 45–60 min.

Localization and Distribution of H1 and H2. By determining the fraction of H1 or H2 that is sensitive to trypsin added to intact cells, we can determine the fraction of total receptor on the surface. In one set of studies, H1- or H2-expressing cells were labeled for 30 min with [^{35}S]cysteine and chased for 2 hr with normal growth medium. Cells were treated with or without trypsin for 20 min at 4°C. Fig. 4*A* shows that cell lines expressing H1 alone (1-7 and 1-13) as well as H1 and H2 (1-7-1) contain an H1 precursor species at 40 kDa and a fully processed band at 46 kDa. As expected for an endoplasmic reticulum precursor, this 40-kDa polypeptide is, in all cases, inaccessible to trypsin and thus represents an internal control for cell breakage during the experiment. In all cases, trypsin caused a specific decrease only in the 46-kDa band, the mature form of H1. Quantitatively, in 1-7, 1-13, or 1-7-1 cells the levels of the 46-kDa bands are reduced 50% by trypsin (average of three experiments: range, 40–60%).

Immunoblotting verified that ≈50% of the 46-kDa mature H1 species is on the cell surface. In both 1-7 and 1-7-1 cells, the level of the 40-kDa H1 precursor is unaffected by digestion of intact cells with trypsin at 5 mg/ml (40 min, 4°C), while the intensity of the mature 46-kDa protein is reduced by half (Fig. 4*A*, four right lanes). Thus, in the cell lines expressing H1 alone, or H1 and H2 together, ≈50% of the fully processed 46-kDa H1 species is located intracellularly and ≈50% is on the cell surface. Our results are in concordance with results reported for H1 and H2 polypeptides in HepG2 cells (6).

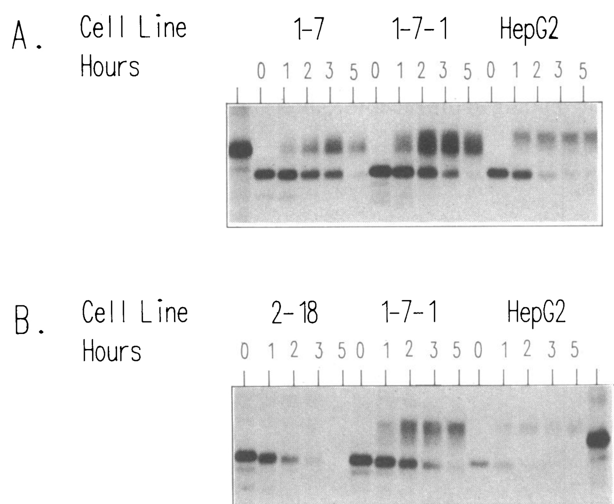

FIG. 3. (*A*) Pulse–chase labeling of the ASGP-R H1 polypeptide. 1-7 cells (lanes 2–6 from the left), 1-7-1 cells (lanes 7–11), and HepG2 cells (lanes 12–16) were labeled for 20 min at 37°C with [^{35}S]cysteine and chased with growth medium for the times indicated. Cells were then solubilized and immunoadsorbed with anti-H1 antibody. The immunoprecipitates were analyzed by SDS/PAGE followed by fluorography. A ^{14}C ovalbumin (44 kDa) size standard is in lane 1. (*B*) Pulse–chase labeling of the ASGP-R H2 polypeptide. Cells that express H2—2-18 (lanes 1–5 from the left), 1-7-1 (lanes 6–10), and HepG2 (lanes 11–15)—were subjected to the same protocols as in *A*, except that anti-H2 antibody was used for immunoadsorption. Lane 16 is the ovalbumin marker.

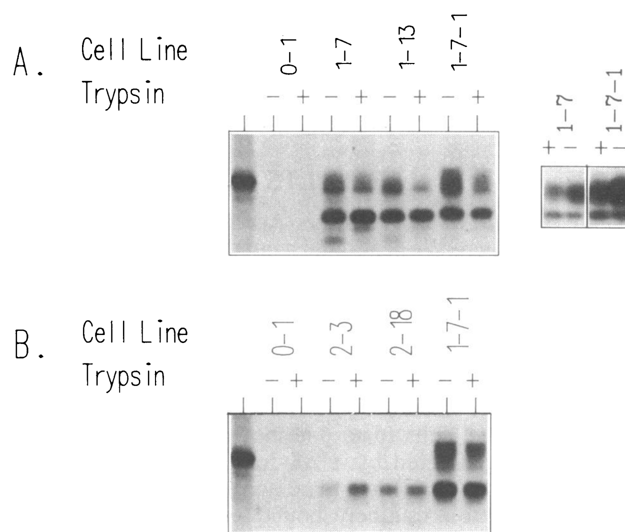

FIG. 4. (*A*) Effect of extracellular trypsin on the amount of cell-associated H1 or H2 polypeptide. Lane 1 (from the left) is the ovalbumin marker. In lanes 2–9 (*A* and *B*) cells were pulse-labeled for 30 min with [^{35}S]cysteine and chased for 2 hr with normal growth medium. The cells were then washed with HBS at 4°C and treated for 20 min at 4°C with trypsin (5 mg/ml)/1 mM EDTA in HBS (lanes +), or with HBS alone (lanes −). The cells were then washed three times with HBS containing soybean trypsin inhibitor (1 mg/ml) and 1% bovine serum albumin. Cells were solubilized, immunoadsorbed with anti-H1 (*A*) or anti-H2 (*B*) antibodies, and subjected to SDS/PAGE and fluorography. In the four right-hand lanes of *A*, unlabeled 1-7 or 1-7-1 cells, treated with or without trypsin for 40 min at 4°C, were washed and solubilized as described above and immunoblotted with anti-H1 antibody.

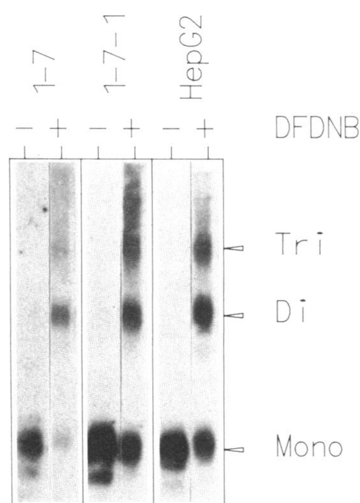

FIG. 5. Immunoblot of cross-linked microsomes with anti-H1 antibody. Microsomal membrane proteins were isolated from 1-7 cells (H1 only), 1-7-1 cells (expressing H1 and H2), and HepG2 cells. These were treated with (lanes +) or without (lanes −) 0.5 mM DFDNB. Solubilized membranes were subjected to SDS/PAGE (7.5% polyacrylamide). The SDS gel was blotted onto nitrocellulose and treated with anti-H1 antibody. Arrowheads indicate presumed receptor monomer, dimer, and trimer species.

Cell lines expressing the H2 polypeptide alone (2-3 and 2-18) exhibit only the 43-kDa labeled H2 precursor. This species is not accessible to trypsin treatment and thus, as expected, is not on the cell surface (Fig. 4*B*). In contrast, in the 1-7-1 cell line, ≈50% of the labeled mature 50-kDa H2 is accessible to trypsin treatment, and therefore 50% is localized to the cell surface. Thus, both H1 and H2 have the same relative distribution in the 1-7-1 and HepG2 cells—≈50% of the mature species is on the cell surface (6). We conclude that coexpression of H1 prevents the degradation of some of the H2 precursor polypeptide, allowing some H2 to migrate to the cell surface.

To confirm that H2 reaches the surface in 1-7-1 cells but not 2-3 or 2-18 cells, we labeled the surface of cells by lactoperoxidase-catalyzed radioiodination. Immunoreactive ^{125}I-labeled H2 molecules are present in 1-7-1 cells but not in 2-18, whereas H1 is labeled by surface iodination in the presence or absence of H2 (data not shown).

Chemical Cross-Linking. Isolated microsomal membranes were reacted with the homobifunctional cross-linker DFDNB to determine whether H1, when expressed alone in 3T3 cells, forms oligomers (Fig. 5). In all cell lines that express the H1 polypeptide (1-7, 1-7-1, and HepG2), DFDNB cross-links H1 to form apparent dimers (≈93 kDa) and trimers (≈148 kDa). We conclude that H1 homotrimers are formed in 1-7 cells. In HepG2 cells, we showed that some cross-linked dimers and trimers that contain H1 also contain H2 (10), and by the same protocol we have demonstrated that this is the case also in the 1-7-1 cells (data not shown).

DISCUSSION

The human ASGP-R, like the rat ASGP-R, is the product of two distinct genes. To dissect the involvement of each polypeptide in ASGP-R function, we have studied the biosynthesis, processing, and activity of each polypeptide in NIH 3T3 fibroblasts expressing H1 alone, H2 alone, or both polypeptides together.

When H1 is expressed alone in NIH 3T3 cells, it is synthesized and inserted into the endoplasmic reticulum, core glycosylated, processed to its 46-kDa mature form, and transported to the cell surface with nearly the same kinetics and efficiency as is H1 in the human hepatoma HepG2 (Figs. 3 and 4). Importantly, the cells are unable to bind to the ligand ASOR. When H2 is expressed alone in NIH 3T3 cells, it is synthesized, inserted into the endoplasmic reticulum, and core glycosylated as its normal 43-kDa precursor, but it is rapidly degraded. The oligosaccharides on H2 are not processed any further than the high-mannose form and most likely the H2 polypeptide never reaches the Golgi (Figs. 3 and 4). Where H2 is degraded is not known; certainly it is not transported to the cell surface and cannot bind ASOR ligand. Coexpression of H1 with H2 "rescues" H2. Some H2 species are fully processed to the mature 50-kDa form and are transported to the cell surface; some are degraded (Figs. 3 and 4). In 1-7-1 cells that express both H1 and H2, all functions of the ASGP-R are indistinguishable from those in the human hepatoma HepG2: K_d of binding of ASOR to cell-surface receptors, and rates of internalization and degradation of ASOR and of recycling of the ASGP-R (Figs. 1 and 2). We conclude that both polypeptides do interact early in biosynthesis and that both polypeptides are required to form the ASGP-R.

Several additional lines of evidence demonstrate that the human ASGP-R is a multichain complex composed of both H1 and H2 polypeptides. In a previous paper (10), we demonstrated that in HepG2 cells H1 and H2 form a hetero-oligomer by showing that the two polypeptides can be specifically cross-linked to each other to form heterodimers and heterotrimers. Also, when HepG2 cells are treated with antibodies directed against the exoplasmic domains of either H1 or H2, a concomitant and equal degradation of both H1 and H2 polypeptides is induced.

Despite the fact that cells that express only H1 on their surface cannot bind the ligand ASOR, individual H1 and H2 polypeptide chains do have some intrinsic galactose-binding activity. When a Triton X-100 extract (including calcium) of cells that express H1 alone is passed over a column of galactose-agarose, all of the H1 polypeptide is specifically retained. The ASGP-R polypeptide can then be specifically eluted by lowering the pH and identified by immunoadsorption or by precipitation with tricholoroacetic acid (J. Bischoff and M.A.S., unpublished observations; data not shown). Drickamer and coworkers (9, 22) obtained a similar result for RHL-1 and RHL-2/-3, when synthesized *in vitro* in the presence of dog pancreatic microsomes. However, the ability of purified ASGP-R polypeptides to bind specifically and efficiently to galactose-agarose does not indicate that a single ASGP-R polypeptide can mediate high-affinity cell-surface binding of a protein bearing a normal galactose-terminal triantennary oligosaccharide. Although 1-7 cells, which express only H1, have about as much H1 on their surface as do 1-7-1 cells, which express both H1 and H2 (see text and Fig. 4), 1-7 cells do not exhibit detectable binding of ^{125}I-ASOR.

Detergent-solubilized H1 or H2 or RHL-1 or RHL-2 polypeptides may bind specifically to galactose-agarose because of the high apparent concentration of galactose residues immobilized on the agarose matrix. In contrast, high-affinity binding ($K_d = 10^{-8}$ to 10^{-9} M) of a ligand to the cell-surface ASGP-R requires that the receptor be in or assume a specific shape so that it can interact simultaneously with at least three galactose residues that are at the termini of the branches of typical N-linked oligosaccharides (23–27). We cannot determine whether H1, expressed without H2 on the cell surface (1-7 cells), can bind single galactose residues on a branched oligosaccharide. We were unable to detect any specific binding of ^{125}I-ASOR to such cells, but if the binding affinity were that of a typical monogalactose oligosaccharide ($\approx 10^{-3}$ M; ref. 24) or even of a typical oligosaccharide with two terminal galactose residues (K_d, $\approx 10^{-6}$ M) we would not have been able to detect such low-affinity specific binding (11).

Thus, our data indicate that the functional ASGP-R is a heterooligomer containing H1 and H2. This hypothesis is supported by observations of McPhaul and Berg on the rat ASGP-R (7), showing that both receptor cDNAs must be expressed in a cell to reconstitute a functional ASGP-R. Our data are not in agreement with the findings of Halberg *et al.* (9) that the rat ASGP-R polypeptides form only homooligomers, but we do agree with the observations of Sawyer *et al.* (8).

We do not know the exact subunit composition of the human ASGP-R. In HepG2 cells, the radiation target size of the functional ASGP-R is 140 kDa (28). This, together with the cross-linking studies detailed in the previous paper (6), indicates that the functional receptor is minimally a primer. The most likely composition would then be $(H1)_2H2$, since in HepG2 cells H1 is produced in excess over H2. In 1-7 cells that synthesize only H1, we can detect H1 dimers and H1 trimers, and thus H1 may reside on the cell surface as a homodimer or homotrimer. However, H1 homooligomers cannot function in high-affinity ligand binding or internalization.

We propose a model for the ASGP-R in which a core of three H1 molecules can noncovalently interact with up to three H2 molecules, while each H2 might be able to interact with one or two H1 molecules. The apparent ratios of H1 to H2 can vary from 1:1 for the 1-7-1 cells, to 2–3:1 for the 1-7-2 cells (data not shown), and 5–6:1 for HepG2 cells (6). In all cases, H1 and H2 together form the active functional receptor, presumably because they differ in ability to bind the three galactose residues in a triantennary oligosaccharide.

Our data also show that the ASGP-R is a member of a class of multipeptide integral membrane proteins whose assembly into their proper tertiary structure is a prerequisite for transport to the cell surface and proper function. Newly made H2 polypeptides are degraded and do not appear to exit the rough endoplasmic reticulum unless the H1 polypeptide is coexpressed. Apparently, association of H1 with H2 is necessary to stabilize the H2 polypeptide and/or to allow it to enter the vesicles that transport proteins from the endoplasmic reticulum to the Golgi. A corollary is that H1 and H2 interact early in biosynthesis, probably in the endoplasmic reticulum. H1, on the other hand, will mature normally to the cell surface whether or not H2 is synthesized; H1 can form a homooligomer that may allow it to exit the endoplasmic reticulum in the absence or presence of H2.

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1. Ashwell, G. & Harford, J. (1982) *Annu. Rev. Biochem.* **51**, 531–554.
2. Breitfeld, P. P., Simmons, C. F., Jr., Strous, G. J., Geuze, H. J. & Schwartz, A. L. (1985) *Int. Rev. Cytol.* **97**, 47–95.
3. Schwartz, A. L. (1984) *CRC Crit. Rev.* **16**, 207–233.
4. Spiess, M., Schwartz, A. L. & Lodish, H. F. (1985) *J. Biol. Chem.* **260**, 1979–1982.
5. Spiess, M. & Lodish, H. F. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6465–6469.
6. Bischoff, J. & Lodish, H. F. (1987) *J. Biol. Chem.* **262**, 11825–11832.
7. McPhaul, M. & Berg, P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8863–8867.
8. Sawyer, J. T., Sanford, J. P. & Doyle, D. (1988) *J. Biol. Chem.* **263**, 10534–10538.
9. Halberg, D. F., Wager, R. E., Farrell, D. C., Hildreth, J., Quesenberry, M. S., Loeb, J. A., Holland, E. C. & Drickamer, K. (1987) *J. Biol. Chem.* **262**, 9828–9838.
10. Bischoff, J., Libresco, S., Shia, M. A. & Lodish, H. F. (1987) *J. Cell Biol.* **106**, 1067–1074.
11. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
12. Korman, A. J., Frantz, J. D., Strominger, J. L. & Mulligan, R. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2150–2154.
13. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33**, 153–159.
14. Deitcher, D. L., Neutra, M. R. & Mostov, K. E. (1986) *J. Cell Biol.* **102**, 911–919.
15. White, B. A. & Bancroft, F. C. (1982) *J. Biol. Chem.* **257**, 8569–8572.
16. Tanabe, T., Pricer, W. E. & Ashwell, G. (1979) *J. Biol. Chem.* **254**, 1038–1043.
17. Schwartz, A. L., Fridovich, S. E. & Lodish, H. F. (1982) *J. Biol. Chem.* **257**, 4230–4237.
18. Schwartz, A. L., Fridovich, S. E., Knowles, B. B. & Lodish, H. F. (1981) *J. Biol. Chem.* **256**, 8878–8888.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. Skinner, M. K. & Griswold, M. D. (1983) *Biochem. J.* **209**, 281–284.
21. Patel, V. P. & Lodish, H. F. (1987) *J. Cell Biol.* **105**, 3105–3118.
22. Hsueh, E. C., Holland, E. C., Cartera, G. M. & Drickamer, K. (1986) *J. Biol. Chem.* **261**, 4940–4941.
23. Connolly, D. T., Townsend, R. R., Kawaguchi, K., Bell, W. R. & Lee, Y. C. (1982) *J. Biol. Chem.* **257**, 939–945.
24. Hardy, M. R., Townsend, R. R., Parkhurst, S. M. & Lee, Y. C. (1985) *Biochemistry* **24**, 22–28.
25. Lee, R. T. & Lee, Y. C. (1987) *Biochemistry* **26**, 6320–6329.
26. Lee, R. T., Lin, P. & Lee, Y. C. (1984) *Biochemistry* **23**, 4255–4261.
27. Lee, Y. C., Townsend, R. R., Hardy, M. R., Lonngren, J., Arnarp, J., Haraldson, M. & Lonn, H. (1983) *J. Biol. Chem.* **258**, 199–202.
28. Schwartz, A. L., Steer, L. J. & Kempner, E. S. (1984) *J. Biol. Chem.* **259**, 12028–12029.