Membrane-bound Versus Secreted Forms of Human Asialoglycoprotein Receptor Subunits

ROLE OF A JUXTAMEMBRANE PENTAPEPTIDE*

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The H2a alternatively spliced variant of the human asialoglycoprotein receptor H2 subunit differs from the H2b variant by an extra pentapeptide, EGHRG, present in the ectodomain next to the membrane-span. This difference causes retention and degradation in the endoplasmic reticulum (ER) of H2a when expressed without the H1 subunit in 3T3 cells (1). In contrast, a significant portion of singly expressed H2b is Golgi-processed and reaches the cell surface. Using a new specific anti-H2a antibody, we found that in H2a-R cells, H2a is rapidly cleaved to a 35-kDa fragment, comprising the entire ectodomain, most of which is secreted into the medium. The cleavage site for the secreted fragment was located at the luminal end of the membrane span. No membrane-bound H2a exits the ER, indicating that the pentapeptide is a signal for ER retention and degradation of the membrane form but does not hinder secretion of the cleaved soluble form. H2a does not form a membrane receptor complex with H1 as H2b does. H2a is therefore not a subunit of the receptor but a precursor for a secreted form of the protein; signal peptidase is probably responsible for the cleavage to the soluble fragment (2). Therefore, the juxtamembrane sequence regulates the function of the transmembrane domain of a type II membrane protein as either a signal-anchor sequence (H2b) or as a cleaved signal sequence, which generates a secreted product (H2a).

In recent years the concept of a unique pair consisting of a membrane receptor with its soluble ligand has been modified by the discovery of soluble forms of receptors and membrane-bound forms of ligands, i.e. soluble tumor necrosis factor receptor (3) and membrane-bound tumor necrosis factor (4). The formation of normal and aberrant soluble forms of a transmembrane protein is the basis for Alzheimer’s disease, where the amyloid precursor protein is cleaved and released a soluble peptide, which is secreted and may form β-amyloid deposits (5). In general little is known about the mechanism and regulation of the formation and function of the soluble forms of transmembrane receptors. The existence of a soluble form of a human asialoglycoprotein receptor (ASGPR)* subunit and its mechanism of formation from an alternatively spliced variant, which we report here, may help shed some light on these matters.

The human ASGPR is exclusively expressed in hepatocytes. It is constructed of two subunits of related amino acid sequence, H1 (46 kDa) and H2 (50 kDa) (6). The polypeptide chains span the membrane once in a type II orientation with a large carboxyl-terminal ectodomain and a small cytoplasmic tail. The transmembrane segment is an uncloned signal anchor sequence (7). Both H1 and H2 undergo phosphorylation on serine or threonine residues and are N-glycosylated. H1 and H2 form a hetero-oligomeric complex. Expression of both H1 and H2 cDNAs is required to generate high affinity asialoglycoprotein binding sites (8), although each subunit has a binding pocket and binds to a galactosylated matrix (9). An average subunit ratio of 3:1, H1:H2 is found on the surface of HepG2 cells (10).

We have described the different subcellular fates of the two alternatively spliced variants of the H2b subunit of the ASGPR, H2a and H2b, when expressed in NIH 3T3 cells without H1. These variants differ only by the presence in H2a of an extra 5-amino acid insert in the ectodomain adjacent to the membrane spanning region (1). Without coexpression of the H1 subunit, H2a was inserted normally into the ER membrane but was retained in and degraded in the ER or another pre-Golgi compartment; none reached the cell surface (11, 12). In contrast, a significant portion of the alternatively spliced form H2b, expressed without H1, was processed by Golgi enzymes and reached the cell surface. The sole difference determining the different subcellular localization of the two variants is the 5-amino acid insert in H2a (EGHRG) (1). Other alternatively spliced forms of H2a and H2b, lacking an 18-amino acid stretch in the cytoplasmic tail, have been described; their fate is similar to that of H2a and H2b, respectively, stressing the importance of the 5-amino acid membrane-adjacent segment in determining subcellular fate (13). Similar mechanisms have been described for other membrane proteins that undergo rapid degradation in the ER. In the case of unassembled a subunit of the T-cell antigen receptor, specific charged residues in the membrane domain are determinants for ER retention and degradation (14). Similarly, the juxtamembrane charged residues...
in the extra pentapeptide of H2a could also be involved in ER retention and degradation of the singly expressed subunit.

In transfected 3T3 cells, H2a, after insertion into the ER membrane, is cleaved just exoplasmic to the membrane-spanning domain, in the region of the 5 extra amino acids, releasing a 35-kDa soluble fragment comprising the entire ectodomain. Our initial studies suggested that this cleavage might be an obligatory first step in ER degradation of the polypeptide (11, 15). However, we recently showed that ER degradation can occur through at least two pathways, one of them not involving this cleavage in the juxtamembrane domain (2).

In HepG2 cells H2a represents about 8% of H2 mRNAs (1). In order to study the H2a and H2b polypeptides in HepG2 cells, we have developed specific anti-peptide antibodies against the region of the extra five amino acids in H2a that are unique to this variant. Using these antibodies we show here that in HepG2 cells the ectodomain of H2a, cleaved off as a 35-kDa fragment in the ER, is then efficiently secreted; the fragment is also partially secreted from transfected 3T3 cells, although in this case most of it undergoes ER degradation, as we described previously (1).

Secreted forms have been found recently for many membrane receptors (16). In some cases cleavage of the wild type receptor occurs at the cell surface (17). In others alternative splicing creates truncated mRNAs encoding proteins without the transmembrane domains that are secreted (18). In the case of the ASGPR H2a, alternative splicing creates a transcript containing an extra mini-exon, which plays a role in the cleavage and secretion of the ectodomain as we describe here. This result strengthens the theory that signal anchor domains on type II transmembrane proteins are functionally equivalent to signal peptides (19, 20).

EXPERIMENTAL PROCEDURES

Materials—Rainbow 14C-labeled methylated protein standards were obtained from Amersham Corp. [35S]Cysteine was from ICN Radiochemicals (Irvine, CA; >80 Ci/mmol). [3H]Leucine was from American (140 mCi/mmol). Immobilon-P paper was from Millipore Corp. (Bedford, MA). Protein A-Sepharose was from Repligen (Cambridge, MA). Endo-N-acetylglucosaminidase H (endo H) was obtained from New England Biolabs (Beverly, MA). N-Glycanase was obtained from Boehringer Mannheim. Sulfoconcanamidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate and immunoperoxidase were obtained from Pierce. Streptavidin-conjugated peroxidase was from Jackson Laboratories (West Grove, PA). Hygromycin B was from Calbiochem (San Diego, CA). Other inhibitors and common reagents were from Sigma.

Cell Lines and Culture—An NIH 3T3 cell line, transfected with a plasmid containing 90-mm tissue culture dishes were rinsed and incubated with 1% Triton X-100 plus 0.5% sodium deoxycholate containing 2 ml oxidized glutathione and 2 ml PMSF. Immunoprecipitates from cell lysates and end H treatments were performed as described before (1, 11). Immunoprecipitates from cell supernatants (1.2 ml from 60-mm dishes) were done directly by addition of 2 ml PMSF, protein A-Sepharose, and the appropriate antiserum followed by incubation at 4°C with rotation for 4–16 h. For treatment with N-glycanase, immunoprecipitates were washed and then boiled in 10 ml of 0.5% SDS in 50 mM sodium citrate, pH 6.0. Then, 10 ml of a solution containing 200 ml sodium phosphate, pH 8.0, 40 ml EDTA, pH 8.0, 3 ml N-acetylglucosamine was added, together with 40 ml of N-glycanase, and incubations were carried out overnight at 37°C. Twenty ml of sample buffer were added and the samples boiled before loading for SDS-PAGE.

Gel Electrophoresis, Fluorography, and Densitometry—SDS-PAGE was performed on 10% Laemmli gels except where stated otherwise. The gels were analyzed by fluorography using 20% 2,5-diphenyloxazole and quantitated by densitometry as described previously (2).

Cell Surface Biotinylation and Detection of Biotinylated Proteins—Secreted cell monolayers in 90-mm tissue culture dishes were rinsed twice in PBS-CM (PBS containing Ca2+ and Mg2+) and incubated in a fresh solution of 0.5 mg/ml sulfoconcanamidyl-6-(biotin-amido)hexanoate in PBS-CM for 45 min at 4°C with rocking. The reaction was quenched by removing the biotin solution and incubating the cells with 50 ml 0.1 M NH4Cl in PBS-CM for 10 min at 4°C. The cells were then rinsed twice with cold PBS-CM and lysed in denaturing conditions, followed by immunoprecipitation and SDS-PAGE. The products were transferred to a nitrocellulose membrane. Blocking was done by incubating the membrane in TGG (Tris-buffered saline containing 10 g/l glyceral, 1 M glucose and 0.5% Tween 20) containing 1% milk and 3% bovine serum albumin for 60 min at 4°C. Then the membrane was incubated in TGG containing 14C-streptavidin conjugated to peroxidase for 60 min at room temperature. Three washes were done in Tris-buffered saline containing 0.5% Tween 20, and detection was performed by autoradiography and then excised for automated Edman degradation with an Applied Biosystem Inc. (Foster City, CA) model 470A protein sequencer. The products of each reaction cycle were quantitated in a liquid scintillation counter.

RESULTS

The H2a Ectodomain Is Secreted from HepG2 Cells and From Transfected 3T3 Cells—In singly transfected 3T3 cells (without H1), H2a is completely retained in and degraded in the ER or a closely related pre-Golgi compartment (1). Both H2a and H2b produce 35-kDa fragments corresponding to their ectodomain (2, 11). In a pulse-chase experiment with [35S] cysteine (Fig. 1), cells expressing H2a produce more of the fragment (immunoprecipitates with anti-H2 carboxyl-terminal antibody) than cells expressing H2b. At its peak level (1 h of chase), there is 25-fold more H2a fragment than H2b fragment as compared to the amount of pulse-labeled precursors. The intracellular fragments then disappear, and we had presumed that they were entirely degraded. Nevertheless, the transient higher abundance of the H2a 35-kDa fragment prompted us to study its fate in HepG2 cells.

In HepG2 cells H2a represents about 8% of total H2 RNAs. In order to study the H2a and H2b polypeptides in these cells, we developed specific anti-peptide antibodies against the region containing the extra 5 amino acids in H2a (as detailed under “Antibodies”). Fig. 2 shows a pulse-chase experiment followed by immunoprecipitation from cell lysates or from cell supernatants. HepG2 cells were compared to 3T3 cells express-
H2a and H2b (full triangles) in all 11 labeled cysteine residues remain after the cleavage. The values for amounts of fragment were corrected, taking into consideration that only 8 of the original 11 labeled cysteine residues remain after the cleavage.

As can be seen in Fig. 2 as well as in other figures, there is a considerable heterogeneity in the SDS-PAGE profiles of both 35-kDa fragment (bands from 28 kDa to 35 kDa), and of the membrane-attached precursor (bands around 42 kDa). This is due to variable degree of occupation of the three possible N-glycosylation sites, as all of these species are converted to two tight bands at 37 kDa (precursor) and 28 kDa (fragment) after treatment with endo H or N-glycanase (Fig. 2, lanes 8 and 9). The two deglycosylated precursor and two fragment bands present in HepG2 cells that are immunoreactive with the anti-H2-COOH antibody (B, lane 3) may represent the respective H2a and H2b species. In HepG2 cells the H2a polypeptide was found mainly in the form of the cleaved 35-kDa fragment. After a 30-min pulse and 1 h of chase (Fig. 2A, lane 1), no full-length precursor H2a was found in HepG2 cells compared to its presence in transfected 3T3 cells (2–18 cells) (Fig. 2A, lane 7). Even after short pulses, only traces of precursor could be seen in HepG2 cells (data not shown), suggesting a cotranslational or very fast posttranslational cleavage. After 6 h of chase, labeled H2a fragment was secreted into the medium, both from HepG2 cells and from 2–18 cells (Fig. 2A, lanes 4 and 10). While the intracellular H2a fragment was sensitive to endo H, indicating a pre-Golgi localization (Fig. 2A, lanes 2 and 8), the secreted fragment was completely resistant to endo H (Fig. 2A, lanes 5 and 11) but sensitive to N-glycanase (Fig. 2A, lanes 6 and 12), indicating that it had undergone Golgi processing to attain complex type oligosaccharides. The Golgi processed fragment could never be detected within the cells, suggesting rapid secretion after exit from the ER.

The Carboxyl Terminus of the H2a Fragment Is Modified before Secretion—Only a tiny fraction of the secreted H2a fragment can be immunoprecipitated by the anti-H2-COOH-terminal antibody (Fig. 2B, lanes 4 and 10), which would explain why we had not identified the fragment in the culture medium previously (1, 2, 11). It was only after N-glycanase treatment that a clear compact band of the secreted fragment could be observed in immunoprecipitates of the medium using the anti-H2-COOH antibody (Fig. 2B, lane 12). Recognition of the H2a fragment by both the anti-H2-COOH antibody and the anti-H2a antibody would indicate that it comprises the whole ectodomain. However, the weak recognition by the anti-H2-COOH antibody would suggest that a carboxyl-terminal modification occurs to the fragment before secretion, as the intracellular form of the fragment is well recognized by this antibody. The intracellular fragment formed in 3T3 cells expressing H2b (Fig. 2B, lanes 14 and 15) could not be detected in the medium (Fig. 2B, lane 18). Although a similar carboxyl-terminal modification could be occurring in the case of H2b, leading to poor immunoprecipitation by the H2-COOH antibody, no secreted fragment was detected in repeated pulse-chase experiments even after overexposing the autoradiograms (data not shown). To determine if this carboxyl-terminal modification was occurring early in the secretory pathway, we pulse-labeled HepG2 cells for 30 min. Cell lysates were first immunoprecipitated with the anti-H2-COOH antibody. The supernatant was then re-immunoprecipitated with the anti-H2-COOH antibody. The supernatant from this second step was then immunoprecipitated with anti-H2a antibody. A strong band is seen at around 35 kDa (Fig. 4A, lanes 3 and 6), which shows that much of the ER (endo H-sensitive) fragment is not recognized by the anti-carboxyl-terminal antibody. In the case of 2–18 cells, a portion of the precursor species at 42 kDa is not recognized by the anti-carboxyl-terminal antibody, but it is immunoprecipitated by the anti-H2a antibody. This could indicate that in these cells, some carboxyl-terminal modification occurs even before the cleavage of precursor H2a to the 35-kDa fragment.

On the other hand, significant fractions of H2a 42-kDa precursor and 35-kDa fragment are recognized by both antibodies, as seen by immunoprecipitating with anti-H2a, boiling the pellet, and then re-immunoprecipitating with anti-H2-COOH (Fig. 4B). This indicates that, at least for some of the H2a polypeptides, the carboxyl-terminal modification occurs after the cleavage to the 35-kDa fragment. Although H2a mRNA accounts for only 8% of H2 mRNA (1), HepG2 cells express comparable amounts of H2a and H2b polypeptides, as determined by the similar abundance of intracellular 35-kDa H2a fragment (Fig. 4A, lane 3) compared to the H2b 42-kDa precursor (Fig. 4A, lane 1).
The recognition of the secreted H2a fragment by the anti-H2a antibody indicated that it contains the 5-amino acid H2a pentapeptide and therefore that the cleavage site must be near the 3 or 4 amino acids located between the transmembrane domain and the pentapeptide. In order to determine the amino terminus of the H2a fragment secreted from HepG2 cells, the cells were labeled with [3H]leucine for 30 min and chased with unlabeled medium for 4 h. The medium was immunoprecipitated with the anti-H2a antibody, treated with N-glycanase, run on SDS-PAGE, and blotted onto Immobilon-P paper. The band corresponding to the deglycosylated H2a fragment (28 kDa) was excised and subjected to microsequencing (Fig. 5). The appearance of radiolabel after cycles 11 and 15 indicates
H2a undergoes a carboxyl-terminal modification that hinders recognition by the anti-carboxyl-terminal antibody. A sequential immunoprecipitation shows that most H2a fragment in HepG2 cells is not recognized by the anti-carboxyl-terminal antibody and indicates the presence of comparable amounts of H2a and H2b. HepG2 cells or the transfected cell lines 2–18 (H2a) and 0–1 (only neo) were metabolically labeled with [35S]cysteine for 30 min. The cells were then lysed and immunoprecipitated with anti-H2-COOH antibodies (treatment A, lanes 1, 4, and 7). The supernatants were re-immunoprecipitated with anti-H2-COOH antibodies (treatment B, lanes 2, 5, and 8), and the supernatants from this second immunoprecipitation were immunoprecipitated with anti-H2a antibodies (treatment C, lanes 3, 6, and 9). All antibodies were cross-linked to protein A-Sepharose as described under “Experimental Procedures.” The immunoprecipitates were analyzed by SDS-PAGE followed by fluorography. On the right are molecular masses of protein standards in kilodaltons. B, some of the H2a molecules can be recognized by both anti-H2a and anti-carboxyl-terminal antibodies. The same cell lines as in A were metabolically labeled with [35S]cysteine for 30 min. The cells were then lysed and immunoprecipitated with anti-H2a antibodies. The immunoprecipitates were then boiled with 1% SDS and 2 μM dithiothreitol in PBS, after which 10 volumes of 1% Triton X-100 plus 0.5% sodium deoxycholate containing 2 μM oxidized glutathione were added and the samples re-immunoprecipitated with anti-H2-COOH antibodies (lanes 1–3). The same procedure was followed but with anti-H2-COOH antibodies first and then anti-H2a antibodies (lanes 4–6). The immunoprecipitates were analyzed by SDS-PAGE followed by fluorography. On the right are molecular masses of protein standards in kilodaltons.
using anti-H2 carboxyl-terminal antibody instead of anti-H2a (data not shown). As can be seen in panel B, secretion of H2a is also unaffected by coexpression of H1 in 3T3 cells as a similar amount of fragment (relative to precursor after the pulse) can be seen secreted from cells expressing H1 and H2a or H2a alone (Fig. 7B, lanes 5 and 6 compared to lanes 9 and 10). No secreted product is detected with the anti-H1 carboxyl-terminal antibody (Fig. 7B, lanes 7 and 8).

H2a Is Expressed Neither on the Surface of HepG2 Cells Nor on That of Transfected 3T3 Cells—No H2a had been found at the surface of singly transfected 3T3 cells (1). To determine if membrane-bound H2a could be found at the surface of HepG2 cells or if any H2a fragment could form part of the cell surface receptor complex, we biotinylated surface proteins of HepG2 cells or the transfected cell lines with the membrane-impermeant reagent sulfo-SUdMH6N-biotin at 4 °C. Cell lysates were then prepared in denaturing conditions and immunoprecipitated. The immunoprecipitates were run on SDS-PAGE and blotted onto nitrocellulose. The blot was reacted with streptavidin-peroxidase and revealed surface expression of H1 in HepG2 cells and 3T3 cells expressing H1 plus H2a (Fig. 8, lanes 1 and 8). H2b surface expression was also revealed in 3T3 cells expressing H2b and on HepG2 cells (Fig. 8, lanes 3–5). In contrast H2a surface expression was absent in all the cell lines (Fig. 8, lanes 2, 6, and 9). Intracellular H2a could be detected if the biotinylation was performed after the cell lysis (data not shown). In the denaturing conditions that were used to prepare the lysates, all H2b was recovered in HepG2 cells after immunoprecipitation of H1, indicating that no complexes remained (Fig. 8, compare lanes 3 and 4). To rule out the possibility that any trace amounts of H2a were on the surface, another experiment was performed in which we surface-labeled HepG2 cells at 4 °C with the membrane-impermeable Bolton-Hunter reagent. When immunoprecipitation of cell lysates from these cells with anti-H2a antibodies, no cell surface H2a could be detected, although the same protocol readily detected cell surface H2b (data not shown). We conclude that no membrane-attached H2a reaches the cell surface, although the soluble ectodomain is freely secreted.

**DISCUSSION**

The ASGPR H2a subunit has been used as a model for studies on ER degradation, due to the fact that when the polypeptide was expressed in transfected 3T3 cells in the absence of the H1 subunit it was retained in the ER, in contrast to its alternatively spliced variant H2b (1, 2, 11). We have now studied the fate of H2a in HepG2 cells with the help of a new antibody that discriminates between H2a and H2b. The amount of H2a polyepitope in HepG2 cells, primarily as the soluble 35-kDa fragment, is comparable to that of H2b (Fig. 4A, lanes 1–3). In contrast, H2b mRNA is about 10 times more abundant than H2a mRNA (1). This would suggest different efficiencies in the translation of H2a and H2b mRNAs, assuming that the immunoprecipitation efficiency was similar for the two antibodies. In HepG2 cells the majority of the H2a polyepitope were recovered as 35-kDa endo H-sensitive fragments, which corresponds to the ectodomain of the polyepitope (Fig. 2A, lanes 1–3; Fig. 9). Only trace amounts of the H2a precursor can be seen after pulse labeling in HepG2 cells, compared to the abundant amount of H2a 42-kDa precursor seen in transfected 3T3 cells and detected with the same antibody (Figs. 2A and 7A). This suggests that in HepG2 cells the H2a 42-kDa precursor is cleaved cotranslationally or very soon after translation. This is consistent with efficient cleavage by signal peptidase.
To our surprise we found that the cleaved 35-kDa ectodomain of H2a is efficiently secreted in the form of a 42–45-kDa Golgi processed polypeptide (Figs. 2 and 6). The fact that the fragment is secreted indicates that the endoproteolytic cleavage does not by itself determine further degradation in the ER. Assuming that the intracellular fragment seen is formed efficiently from its 42-kDa precursor (Fig. 4A), the secretion of H2a from HepG2 cells is as efficient as the extent of surface expression of newly made H1 or H2b: about 70% (Fig. 6). In contrast, the maximal extent of secretion of the 35-kDa H2a fragment from transfected fibroblasts (~30%) is relatively inefficient, as is the surface expression of newly made H1 or H2b polypeptides synthesized in singly transfected 3T3 cells (40% and 30%, respectively; Refs. 1 and 8). This could suggest that hetero-oligomers of H1/H2a precursors initially form in the ER in HepG2 cells, and that formation of these hetero-oligomers generates some protection from ER degradation. There are many examples of proteins that become targeted to ER degradation if they cannot assemble into normal oligomers. The immunoglobulin k light chain is secreted only when the heavy chain is coexpressed (22). IgM is secreted only when its subunits are correctly assembled, while monomeric IgM polypeptides are targeted for ER degradation (23, 24). Nevertheless, coexpression of H1 did not lead to a faster cleavage or more efficient secretion of H2a fragment from 3T3 cells (Fig. 7). Metabolic labeling and immunoprecipitation with anti-H1 antibodies of cell lysates or supernatants showed no co-immunoprecipitation of H2a. Only a minor fraction of precursor H1 might co-immunoprecipitate with anti-H2a antibodies (Fig. 7A). Thus the differences in the efficiency of H2a cleavage and secretion between HepG2 cells and transfected 3T3 cells appear to be only related to the difference in cell type. Our new specific anti-H2a antibody recognizes H2a and not H2b (Fig. 2). The recognition of the H2a secreted fragment by both the anti-H2-COOH antibody and the anti-H2a antibody (Fig. 2) and the identification of its amino terminus (Fig. 5) would indicate that it comprises the entire H2a ectodomain. However, the weak recognition by the anti-H2-COOH antibody suggests a carboxyl-terminal modification of the 35-kDa fragment before it is secreted (Figs. 4A and 9). This modification could involve conformational changes or proteolytic trimming.

Although there is no apparent change in the molecular mass of the deglycosylated fragment upon its secretion. This COOH-terminal modification explains why we could not detect any secreted fragment before we had generated the “anti-H2a” antibody. It could be argued that ectodomains from H2b molecules are in fact secreted but cannot be recognized by the anti-carboxyl-terminal antibody because the same COOH-terminal modification has taken place. Due to the smaller amount of intracellular H2b fragment, even if it is secreted, the extent of secretion (relative to the amounts of 42-kDa precursors, Fig. 1) would be much less than for H2a.

H2a never leaves the ER as an intact membrane-bound protein, whether or not H1 is coexpressed. Similarly, H2a cannot be detected on the cell surface, whether or not H1 is coexpressed (Fig. 8). We conclude that H2a is not a subunit of the asialoglycoprotein receptor complex. The only function of the 42-kDa membrane-associated H2a precursor seems to be the generation of the soluble secreted fragment. The fact that membrane-bound H2a does not exit the ER, even in transfected 3T3 cells where substantial amounts of the 42-kDa precursor accumulate (Figs. 2 and 7), would indicate that the extra pentapeptide in H2a can function as a signal for ER retention and degradation when present in the membrane-bound 42-kDa form but does not hinder secretion when present on the cleaved...
35-kDa fragment. The charged sequence, EGHRG, was not found in other membrane proteins. Nevertheless, it has been found for other proteins that charged residues in the membrane domain and its flanking residues can target them for the ER (14, 25).

It was recently reported that a mixture of monoclonal anti-ASGPR antibodies revealed immunoreactive bands on a Western blot from samples of human serum (26). Although in that report it was not elucidated if the proteins were H1, H2a, or H2b, the molecular mass of approx 40 kDa would be consistent with our finding for secreted H2a fragment (fig. 2). Our own preliminary results show the existence of the H2a fragment and not of H1 in normal human serum.2 Many other membrane receptors have been shown to have soluble, secreted forms (reviewed in Ref. 16). Some examples are receptors for: IgA (27), insulin-like growth factor II (mannose 6-phosphate) (28), interleukin-2 (29), epidermal growth factor (30), and interferon growth factor II (31). Some examples are receptors for: IGF, insulin-like growth factor (30), and interferon growth factor II (mannose 6-phosphate) (28), interleukin-2 (29), IgE receptor (FCεRI) (32) of other type II transmembrane proteins caused the protein to be cleaved in thejunction membrane region. A deletion of the cytoplasmic tail of H1 rendered the polypeptide sensitive to cleavage by signal peptidease (35). In the case of other type II membrane receptors that generate soluble forms, signal peptidase does not seem to be involved. For example the low affinity IgG receptor (FcεRII) gives rise by proteolysis to several secreted fragments of the ectodomain (36). The transferrin receptor has a secreted form comprising its ectodomain and the cytoplasmic tail of H1; this protein is secreted (Fig. 5). Shortening of the cytoplasmic tail of H1 rendered the polypeptide sensitive to cleavage by signal peptidase (35). In the case of other type II membrane proteins, the protein is cleaved at the site of the signal peptidease cleavage site (37). This cleavage site is not targeted to ER degradation.

Therefore in HepG2 cells H2a generates only a soluble, secreted proteolytic fragment corresponding to its ectodomain, while H2b, the variant lacking the charged, membrane adjacent pentapeptide associates with H1 to form the functional plasma membrane receptor (Fig. 9). The function of the secreted fragment, its state of oligomerization, and the regulation of its secretion are unknown. Recombinant truncated fragments of the subunit 1 of the rat ASGPR (which were similar or much shorter than the H2a secreted fragment) were able to bind to a galactosylated matrix (38). Therefore we could speculate that the secreted H2a ectodomain may function in binding asialoglycoproteins in the plasma and preventing their interaction with the hepatocyte cell surface receptor, thus prolonging their lifetime in the circulation. On the other hand, its function may be totally unrelated to the function of the membrane receptor, as has been shown for a soluble form of the low density lipoprotein receptor that triggers a response to viral infections (39).

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