Export from the Endoplasmic Reticulum Represents the Limiting Step in the Maturation and Cell Surface Expression of the Human δ Opioid Receptor*

Received for publication, November 29, 1999, and in revised form, February 8, 2000

Ulla E. Petjä-Repo‡§, Mireille Hogue‡, André Laperrière‡, Philippe Walker§, and Michel Bouvier‡¶

From the ‡Département de Biochimie, Université de Montréal, Montréal, Quebec H3C 3J7, Canada and the §AstraZeneca R & D Montréal, St. Laurent, Quebec H4S 1Z9, Canada

Synthesis and maturation of G protein-coupled receptors are complex events that require an intricate combination of processes that include protein folding, post-translational modifications, and transport through distinct cellular compartments. Relatively little is known about the nature and kinetics of specific steps involved in these processes. Here, the human δ opioid receptor expressed in human embryonic kidney 293S cells is used as a model to delineate these steps and to establish the kinetics of receptor synthesis, glycosylation, and transport. We found that the receptor is synthesized as a core-glycosylated Mr 45,000 precursor that is converted to the fully mature Mr 55,000 receptor with a half-time of about 120 min. In addition to trimming and processing of two N-linked oligosaccharides, maturation involves addition of O-glycans containing N-acetylgalactosamine, galactose, and sialic acid. In contrast to N-glycosylation, which is initiated cotranslationally and is completed when the protein reaches the trans-Golgi network, O-glycosylation was found to occur only after the receptor exits from the endoplasmic reticulum (ER) and was terminated as early as the trans-Golgi cisternae. Once the carbohydrates are fully processed and the receptor reaches the trans-Golgi network, it is transported to the cell surface in about 10 min. The exit from the ER was found to be the limiting step in overall processing of the receptor. This indicates that early events in the folding of the receptor are probably rate-limiting and that receptor folding intermediates are retained in the ER until they can adopt the correct conformation. The overall low efficiency of receptor maturation, less than 50% of the precursor being processed to the fully glycosylated protein, further suggests that only a fraction of the synthesized receptors attain properly folded conformation that allows exit from the ER. This indicates that folding and ER export are key events in control of receptor cell surface expression. Whether or not the low efficiency of the ER export is a general feature among G protein-coupled receptors remains to be investigated.

† This work was supported by grants from the Medical Research Council of Canada (to M. B.) and from the Ella and Georg Ehrnrooth Foundation, the Helsinki University Pharmacy, and the Oulu University Scholarship Foundation (to U. P.-R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Scientist of the Medical Research Council of Canada. To whom correspondence should be addressed: Département de Biochimie, Faculté de Médecine, Université de Montréal, P.O. Box 6128, Succursale Centre-Ville, Montréal, Quebec H3C 3J7, Canada. Tel.: 514-343-6319; Fax: 514-343-2210; E-mail: Bouvier@bcm.umontreal.ca.

G protein-coupled receptors (GPCRs)† constitute a true superfamily of membrane proteins with more than 1000 individual members. A variety of chemical messengers, such as neurotransmitters and hormones, act on these receptors, and they also serve as sensory molecules in the olfactory and visual systems. Although overall they do not share extensive sequence identity, they are believed to have a similar three-dimensional structure that is characterized by seven hydrophobic transmembrane domains connected by alternating intracellular and extracellular loops (1–5). The N terminus is extracellular, whereas the C terminus lies within the cytosol. Several post-translational modifications, most notably glycosylation, phosphorylation, and palmitoylation, also have an impact on the structure and function of these important membrane proteins.

The GPCRs are expressed at very low levels in natural tissues and cells, and thus progress in biochemical characterization of these proteins has been greatly facilitated by the use of heterologous expression systems. Over the last few years, this approach has been used to address a number of fundamental questions related to both structural and functional features of these proteins. In some cases, however, expression of wild type (6, 7) or mutant (8–11) receptors in transfected cells has resulted in low levels of functional receptors at the cell surface. Similarly, a number of inherited disorders have been traced to mutations within the GPCRs that result in proteins with reduced cell surface expression (12–16). In many cases this was proposed to be due to misfolding and/or deficient targeting of the protein. Recently, a few GPCRs, such as olfactory receptors (17), visual opsins (18–20), the calcitonin gene-related peptide receptor, and the adrenomedullin receptor (21), were shown to need special chaperone/escort proteins to be expressed at the cell surface. In the case of the γ-aminobutyric acid type B-1 receptor, heterodimerization with another GPCR, the γ-aminobutyric acid type B-2 receptor, was shown to be required (22). In general, however, very little is known about how the GPCRs assume their correct conformation and acquire their covalent modifications and are trafficked from the endoplasmic reticulum (ER) to the plasma membrane.

In an attempt to delineate the basic steps involved in the biosynthesis, maturation, post-translational modifications and

* The abbreviations used are: GPCRs, G protein-coupled receptor; BFA, brefeldin A; BSA, bovine serum albumin; DDM, n-dodecyl-β-D-maltoside; DMEM, Dulbecco’s modified Earl’s medium; Endo H, endo-β-N-acetylglucosaminidase H; ER, endoplasmic reticulum; hOHR, human δ opioid receptor; HEK-293S, human embryonic kidney 293S; NHS, N-hydroxysuccinimide; PBS, phosphate-buffered saline; PNGase F, peptide-N-glycosidase F; PAGE, polyacrylamide gel electrophoresis; SNC-80, (5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl-N, N-diethyl-ylenzaminid; STI, soybean trypsin inhibitor; WGA, wheat germ agglutinin.
targeting of these proteins, the human δ opioid receptor (hδOR) was used as an experimental model. This receptor, along with the other opioid receptors, µ and κ, mediates the actions of endogenous opioid peptides as well as opiate alkaloids in the nervous system (23, 24). The deduced primary structure of the hδOR predicts a protein of 372 amino acids with two consensus N-glycosylation sites in its N-terminal extracellular domain and a calculated weight of approximately 40,000 (25, 26). To date no study has directly investigated the steps leading from the newly synthesized polypeptide to the mature receptor at the plasma membrane. Here, using a heterologous expression system, we studied the rates of biosynthesis, maturation, and metabolic turnover of the hδOR in discrete cellular compartments in relation with the glycosylation events that occur during the transit of the protein from the ER to the cell surface. We found that protein export from the ER represents the rate-limiting step in the processing of the newly synthesized receptor.

**EXPERIMENTAL PROCEDURES**

**Materials**—EPR®-S/5'S protein labeling mix (1175 Ci/mmol), [9-3H(N)]bremazocine (26.6 Ci/mmol), [2,8-3H]adenine (31.7 Ci/mmol), Tunicamycin, wheat germ agglutinin (WGA)-agarose, anti-FLAG M2 (EC 3.2.1.18), monensin, and brefeldin A (BFA) were from Calbiochem. Roche Molecular Biochemicals. Neuraminidase from *Neisseria meningitidis* (EC 3.5.1.52), and 3-isobutyl-1-methylxanthine, 100 μM forskolin, and SNC-80 at a final concentration of 1 mM to 1 μM. The reaction was stopped by aspirating the medium and adding 1 ml of ice-cold 5% trichloracetic acid (w/v). After adding 100 μl of 5 mM ATP/5 mM cAMP, cells were lifted by a rubber policeman, and cellular proteins were removed by centrifugation (700 × g, 10 min). [3H]ATP and [3H]cAMP were measured by the supernatant following separation by sequential chromatography on DEAE-cellulose described (31). Final-binding data was performed using the GraphPad Prism program version 2.01.

**DNA Constructs and Site-directed Mutagenesis**—The hδOR cDNA was subcloned into the pcDNA3 expression vector (Invitrogen) as described (27). The receptor was tagged at the C-terminal end with the FLAG epitope (DYKDDDDK) using the CLONTECH site-directed mutagenesis kit that is based on the method of Deng and Nickoloff (28). The construction was verified and confirmed by restriction enzyme mapping and DNA sequencing.

**Cell Culture and Transfection**—Human embryonic kidney 293S (HEK-293S) cells were grown and maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum, 100 units/ml penicillin and streptomycin, and 1.5 mg/ml amphotericin B, and stored at

**Biosynthesis of the δ Opioid Receptor**

**Radioligand Binding Assays**—Cells were harvested in phosphate-buffered saline (PBS) at 80–90% confluency, quick frozen in liquid nitrogen, and stored at −80 °C. After thawing, the cell pellets were suspended in buffer A (25 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 2 mM EDTA, 5 μg/ml leupeptin, 5 μg/ml soybean trypsin inhibitor (STI), and 10 μg/ml benzonase) and homogenized with a Polytron homogenizer (Ultra-Turrax, T25, Janke and Kunkel) using three bursts of 5 s at maximum setting. Homogenates were centrifuged at 700 × g for 5 min, and the pellets were rehomogenized and recentrifuged. The combined supernatants were further centrifuged at 27,000 × g for 20 min, and the pellets were washed twice with buffer A. The pellets containing the crude membrane fractions were resuspended in buffer A at a concentration of 1–5 mg/ml and either used immediately or quick frozen in liquid nitrogen and stored at −80 °C. Protein was assayed by using the Bio-Rad DC assay kit with bovine serum albumin (BSA) as a standard.

Satisfaction and competition binding assays for the wild type and the FLAG-tagged hδOR were carried out using 5 μg of membrane protein in a final volume of 300 μl of buffer A supplemented with 0.1% BSA (w/v). For saturation experiments, triplicates contained 0.2–20 nM [3H]bremazocine. For competition experiments, triplicates contained 1 nM [3H]bremazocine and either SNC-90, Leu-enkephalin, or naloxone at final concentrations ranging from 0.1 pM to 100 μM. Non-specific binding was determined using 10 μM naltrexone. After incubation at 25 °C for 60 min, the membranes were harvested by rapid filtration over Whatman GF/C filters pre-soaked for 60 min in 0.1% polyethyleneimine (w/v) and washed three times with cold 25 mM Tris-HCl, pH 7.4. The radioactivity in the filters was determined by harvesting in UniverSol™ scintillation fluid (ICN). Curve fitting and analysis of the binding data were performed using the GraphPad Prism program version 2.01.

**CAMP Accumulation Assay**—Intracellular cAMP levels were determined by measuring the conversion of [3H]ATP to [3H]cAMP following metabolic labeling with the [3H]adenine, as described (30). Stably transfected HEK-293S cells were cultured into 6-well culture plates at a density of 100,000 cells/well and allowed to recover for 48 h before the experiments. The cells were incubated at 37 °C for 16 h in complete DMEM containing 2 μCi/ml of [3H]adenine, washed twice with PBS, and incubated further for 15 min in medium supplemented with 1 mM 3-isobutyl-1-methylxanthine, 100 μM forskolin, and SNC-80 at a final concentration of 1 mM to 1 μM. The reaction was stopped by aspirating the medium and adding 1 ml of ice-cold 5% trichloracetic acid (w/v). After adding 100 μl of 5 mM ATP/5 mM cAMP, cells were lifted by a rubber policeman, and cellular proteins were removed by centrifugation (700 × g, 10 min). [3H]ATP and [3H]cAMP were measured by the supernatant following separation by sequential chromatography on DEAE-cellulose described (31). Final-binding data was performed using the GraphPad Prism program version 2.01.

**Saturation and Competition Binding Assays**—Cells were harvested in phosphate-buffered saline (PBS) at 80–90% confluency, quick frozen in liquid nitrogen, and stored at −80 °C. After thawing, the cell pellets were suspended in buffer A (25 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 2 mM EDTA, 5 μg/ml leupeptin, 5 μg/ml soybean trypsin inhibitor (STI), and 10 μg/ml benzonase) and homogenized with a Polytron homogenizer (Ultra-Turrax, T25, Janke and Kunkel) using three bursts of 5 s at maximum setting. Homogenates were centrifuged at 700 × g for 5 min, and the pellets were rehomogenized and recentrifuged. The combined supernatants were further centrifuged at 27,000 × g for 20 min, and the pellets were washed twice with buffer A. The pellets containing the crude membrane fractions were resuspended in buffer A at a concentration of 1–5 mg/ml and either used immediately or quick frozen in liquid nitrogen and stored at −80 °C. Protein was assayed by using the Bio-Rad DC assay kit with bovine serum albumin (BSA) as a standard.
Pharmacological comparison of the wild-type hOR and the FLAG epitope-tagged hOR expressed in HEK-293S cells

Ligand binding assays were carried out using membranes prepared from stably transfected HEK-293S cells expressing the wild type hOR or the FLAG epitope-tagged hOR, whereas cAMP accumulation assays were performed using intact cells. Saturation binding assays were performed using 0.2–20 nM [3H]bremazocine to obtain the Kd and Bmax values. Ki values were determined by competition of 1 nM [3H]bremazocine with the indicated unlabeled opioid ligands (0.1–10 μM). The ligand was added to the resin equilibrated in buffer E supplemented with 0.1% BSA (w/v) was carried out to obtain the IC50 values. Analysis of the data was performed using the GraphPad Prism program, version 2.01. Data represent the means ± S.E. of three to five independent experiments performed in triplicate. There were no statistical differences between the values for the wild type and the epitope-tagged hOR for any parameter.

| Receptor     | Ligand binding | [3H]Bremazocine | cAMP accumulation |
|--------------|----------------|----------------|-------------------|
|              | Kd (nM)        | Bmax pmol/mg protein | SAC-80 μg/ml |
| hOR          | 88.1 ± 9.7     | 2.27 ± 0.43     | 0.76 ± 0.12       | 94.0 ± 1.3     | 2.42 ± 0.3     | 1.25 ± 0.87     |
| hOR-FLAG     | 99.4 ± 5.4     | 2.29 ± 0.27     | 0.73 ± 0.09       | 1.18 ± 0.26    | 23.2 ± 2.3     | 1.36 ± 0.74     |

a Equilibrium inhibition constant.
b Equilibrium dissociation constant.
c Maximal specific binding.
d IC50, half-maximal effective inhibitory concentration.

(see below) that recognizes the native C-terminal end of the receptor was not suitable for immunoprecipitation. 20 μl of antibody-coupled resin equilibrated in buffer E supplemented with 0.1% BSA (w/v) was used to capture the receptor from one 25-cm2 flask. The resin was incubated with the supernatant overnight at 4 °C with gentle agitation, pelleted, and washed twice with 500 μl of buffer F and four times with 500 μl of buffer G containing 0.1% BSA (w/v) for 20 min at 4 °C. The enzymes were added at final concentrations of 25 milliunits/ml (Endo H), buffer H containing 1% 2-mercaptoethanol (PNGase F), or 50 mM sodium phosphate, pH 6.0, 0.5 mM PMSF, 5 μM leupeptin, 5 μg/ml STI, and 10 μg/ml benzamidine (neuraminidase and O-glycosidase).

**RESULTS**

Functional Characterization of the hOR Expressed in HEK-293S Cells—The FLAG epitope was introduced to the C-terminus of the receptor, allowing the labeling of cells. For Western blotting, the proteins resolved in SDS-PAGE were transferred electrochemically from the gels onto nitrocellulose (Amersham Pharmacia Biotech) (50 mA, 16 h, Bio-Rad Mini-Trans Blot apparatus). The nitrocellulose-bound proteins were probed with either a polyclonal receptor antibody directed against a peptide (SDGPPGGRAA) corresponding to the amino acids at the native C-terminal end of the receptor (a generous gift from Dr. Robert Elde, University of Minnesota) or a monoclonal anti-FLAG M2 antibody (Sigma) recognizing the FLAG epitope tag at the C terminus of the receptor, according to the protocol described by the manufacturer of the latter antibody. Horseradish peroxidase-conjugated secondary antibodies from Amersham Pharmacia Biotech, and enhanced chemiluminescence Western blotting detection reagents from NEN Life Science Products were used to reveal the labeled proteins. The electroblotted molecular weight markers were detected by staining with Ponceau S (Sigma).
Identification of the hδOR Species Expressed in HEK-293S Cells—To identify the FLAG epitope-tagged hδOR expressed in HEK-293S cells, the cells were homogenized, and DDM-solubilized membranes were subjected to SDS-PAGE and immunoblotting (Fig. 1A). Anti-FLAG M2 antibody recognized a broad, heterogeneous band with an apparent molecular weight of 55,000 and a sharper one of 45,000 (Fig. 1A, lane 2). The same polypeptides were also detected by a polyclonal antiserum that binds avidly to WGA (Fig. 1A, lane 4). These two bands were not present in nontransfected HEK-293S cells (Fig. 1A, lanes 1 and 3), indicating that they specifically represent the expressed hδOR. Occasionally, higher molecular weight species were detected that could represent dimeric forms of the receptor (33–35).

The identities of the two receptor species were further studied by subjecting the solubilized receptor to purification on WGA-agarose (Fig. 1B). Only the higher molecular weight receptor species was specifically bound to and eluted from the resin with 0.3 M N-acetylglucosamine (Fig. 1B, lane 3). This indicates that the M₅₅,₀₀₀ receptor species is a glycoprotein and demonstrates that it represents the full-length receptor polypeptide. This is based on the facts that the only putative N-glycosylation sites are located in the N-terminal portion of the receptor (Asn-18 and Asn-33) and that the epitope recognized by the antibody is localized in the C-terminal end of the protein. Two possibilities can be envisioned to explain why the M₅₅,₀₀₀ species does not represent a degradation product lacking carbohydrate moieties required for high affinity lectin binding. First, this species could be a degradation product of the M₅₅,₀₀₀ receptor form lacking carbohydrate moieties required for high affinity lectin binding. Second, it could be a full-length receptor containing oligosaccharides that have low affinity for WGA, such as unprocessed high mannose type glycans (36).

To investigate further the latter possibility that the two molecular forms of the hδOR expressed in HEK-293S cells contain the same polypeptide backbone but differ in their carbohydrate structures, the solubilized receptors were treated with glycosidases (Fig. 2). Endo H selectively removes unprocessed high mannose type oligosaccharides from glycoproteins but does not cleave complex type fully processed glycans (37). Treatment of the solubilized proteins with this enzyme did not change the molecular weight of the M₅₅,₀₀₀ receptor species but reduced that of the M₄₅,₀₀₀ one to about M₄₁,₀₀₀ (Fig. 2A, lane 2). This demonstrates that the M₅₅,₀₀₀ receptor species does not represent a degradation product lacking N-glycosylation sites but rather a full-length receptor bearing high mannose-type oligosaccharides.

The resistance of the M₅₅,₀₀₀ receptor species to Endo H treatment (Fig. 2A, lane 2) coupled to the observation that it binds avidly to WGA (Fig. 1B, lane 3) suggests that this species contains complex-type N-linked oligosaccharides. This was fur-
ther supported by the observation that PNGase F, an enzyme that can remove all types of N-linked oligosaccharides from glycoproteins (37), was able to digest the M₄ 55,000 receptor (Fig. 2A, lane 4). The broadness of the band and the occasional detection of discrete species around M₄ 55,000 most likely represent N-glycosylation heterogeneity because this heterogeneity was abolished by the PNGase treatment. As anticipated, PNGase F was also able to increase the electrophoretic mobility of the M₄ 45,000 receptor form (Fig. 2A, lane 4). Interestingly, however, deglycosylation of the two receptor species by this enzyme led to the formation of two distinct molecular weight species of 43,000 and 39,000 respectively. Provided that complete enzymatic cleavage of N-linked carbohydrates was achieved, as indicated by the PNGase F concentration series presented in Fig. 2B, these data suggest that the higher molecular weight receptor species has an additional modification that changes its electrophoretic mobility on SDS-PAGE. The nature of this modification will be addressed in a later section.

Concerning the glycosylation, the difference between the apparent molecular weight of the major hOR species estimated from SDS-polyacrylamide gels (55,000) and the predicted polypeptide size (about 40,000) indicates that both putative N-glycosylation sites of the receptor are occupied by carbohydrate. This was confirmed by stepwise, concentration-dependent digestion of the solubilized receptor by PNGase F from the M₄ 55,000 species to the M₄ 43,000 product via a M₄ 50,000 intermediate form that most likely represents a receptor with a single N-glycan (Fig. 2B). Increasing the enzyme concentration up to 20 units/ml did not decrease the molecular weight of the M₄ 43,000 band any further, indicating that the reaction was complete.

To further investigate the nature of the M₄ 55,000 and the M₄ 45,000 receptor species observed on Western blots, we assessed their cellular location (i.e. intracellular versus cell surface). Intact cells were labeled with a membrane impermeable biotinylation reagent, sulfo-NHS-biotin, and cellular membranes were isolated. After solubilization, the biotinylated proteins were purified by immobilized streptavidin, and the receptor was revealed on a Western blot using the anti-FLAG M2 antibody (Fig. 3). The selectivity of the cell surface biotinylation assay was confirmed by the absence of calnexin, an ER resident transmembrane protein, from the streptavidin-purified fraction (data not shown). This indicates that the sulfo-NHS-biotin does not have access to the intracellular compartments in the conditions used. As shown in Fig. 3 (lane 6), the M₄ 55,000 receptor species was purified by streptavidin-agarose, suggesting that this receptor form is expressed at the cell surface. In contrast, the M₄ 45,000 receptor did not bind to streptavidin and thus is confined to an intracellular compartment that is inaccessible to the extracellular sulfo-NHS-biotin. Taken together, these observations indicate that the M₄ 55,000 hOR species may represent the fully mature receptor form that is transported to the cell surface, whereas the M₄ 45,000 is a precursor form that is found in an intracellular compartment, most likely in the ER.

**Biosynthesis, Processing, and Trafficking of the Newly Synthesized hOR**—To further confirm the potential precursor-product relationship between the two hOR species expressed in HEK-293S cells, pulse-chase experiments were performed (Fig. 4). Cells were labeled for 60 min in a methionine/cysteine-free medium containing 150 μCi/ml of [35S]methionine/cysteine and subsequently chased for periods of up to 22 h in a medium containing an excess of unlabeled methionine. At each time point, cells were harvested and membranes were prepared. The solubilized receptors were immunoprecipitated and subjected to SDS-PAGE followed by fluorography. As shown in Fig. 4A, the different receptor forms do not appear simultaneously but rather in a specific sequence and with quite different kinetics. The M₄ 45,000 band was the first component to be labeled along with two minor ones with apparent molecular weights of about 42,000 and 39,000. None of these three bands was apparent in nontransfected HEK-293S cells (data not shown). During the subsequent chase, label incorporated into these bands decreased in parallel with the appearance of a slower, more diffusely migrating one of the M₄ 55,000, confirming the precursor-product relationship between the M₄ 45,000 and the M₄ 55,000 hOR species and the fact that the latter receptor species corresponds to the fully mature form of the receptor.

The kinetic relationships between the different hOR species were assessed by densitometric scanning of autoradiograms to construct progress curves (Fig. 4C). As can be seen, addition of the terminal sugars reached completion with a half-time of about 120 min (121 ± 4 min). The half-life for the M₄ 55,000 mature receptor species was approximately 15 h (15.3 ± 2.7 h), whereas the turnover rate for the smaller receptor forms was substantially faster (M₄ 45,000, 134 ± 5 min; M₄ 42,000, 99 ± 5 min; and M₄ 39,000, 35 ± 4 min). The M₄ 42,000 receptor form most likely represents a partially glycosylated form of the receptor, whereas the M₄ 39,000 one is the nonglycosylated polypeptide. Indeed, like the M₄ 45,000 form, the M₄ 42,000 species was found to be sensitive to both Endo H and PNGase F treatments leading to a form that comigrates with the M₄ 39,000 species (see Fig. 7, lane 1, in A, B, and C). No precursor-product relationships could be established between the M₄ 39,000, the M₄ 42,000, and the M₄ 45,000 forms, even when the pulse was shortened to 15 min (data not shown), leaving the possibility open that they may represent either incompletely translocated and glycosylated species or deglycosylated forms of the receptor precursor that are destined to a degradation pathway (38–41). The relatively long half-time for the conversion of the precursor to the mature form (120 min) suggests that export from the ER is a limiting step in the overall maturation process of the receptor.

One apparent fact of hOR biosynthesis seen in Fig. 4 (A and C) is that the efficiency of maturation of the receptor is rather low. The fractional conversion of precursor to the mature form was less than 50% (40.5 ± 3.5%, a mean of eight experiments) of maximum label incorporated into the receptor precursor.
Deglycosylation did not increase the intensity of the band corresponding to the mature receptor (see Fig. 7, compare lane 4 in A and C), ruling out the possibility that the amount of the M, 55,000 receptor species was underestimated as a consequence of glycosylation heterogeneity. It could be argued that the inefficient conversion of the receptor to the mature form is an artifact of the high level of expression achieved in HEK-293S cells (~24 pmol/mg protein). However, this is unlikely because a similar low efficiency (39.5%) of maturation was observed when the kinetics of receptor turnover was studied in cells expressing a lower level of receptors (3 pmol/mg protein). Although this expression level is still supraphysiological, the data allow us to argue that the inefficient conversion to a form bearing complex type N-linked oligosaccharides is an intrinsic property of the hOOR polypeptide and does not reflect congestion of a saturable process. Such a saturation would have lead to a lower efficiency in cells expressing higher number of receptors.

To define the time course over which newly synthesized receptors are transported to the cell surface, cells were subjected to biotinylation following different times of chase in the pulse-chase labeling experiments (Fig. 4B). [35S]methionine/cysteine-labeled receptors that had reached the plasma membrane were distinguished from the total pool of labeled receptors by their ability to undergo biotinylation when intact cells were incubated with sulfo-NHS-biotin. The biotinylated cell surface proteins were then isolated by streptavidin-agarose, and the receptors were purified by immunoprecipitation. As can be seen from Fig. 4 (compare panels A and B), the mature M, 55,000 receptor form was the predominant one at the cell surface and was very stable (t½ = 19.6 ± 2 h). It accumulated at the cell surface with a half-time of 134 ± 3 min. Because the half-time for the maturation of the receptor was about 120 min (Fig. 4C), there does not seem to be any lag time between final maturation and transport from the Golgi to the cell surface.

O-Glycosylation of the hOOR—As mentioned previously, removal of N-linked oligosaccharides from the precursor and the mature forms of the hOOR led to species with distinct electrophoretic mobilities (M, 39,000 and M, 43,000 for the precursor and the mature forms, respectively; Fig. 2A). This indicates that the hOOR undergoes an additional post-translational processing step during its transit along the secretory pathway prior to its transport to the cell surface. In addition to N-glycosylation, two other modifications, namely O-glycosylation and phosphorylation, have been shown to decrease the apparent mobility of membrane-bound proteins when analyzed by SDS-PAGE (42–44). The possible contribution of phosphorylation as an explanation for our findings was ruled out by the inability of alkaline phosphatase treatment to increase the mobility of the deglycosylated M, 43,000 hOOR (data not shown). Thus, addition of oligosaccharides to serine or threonine acceptor sites on the receptor is the most probable cause for the additional decreased electrophoretic mobility of the hOOR.

To test the hypothesis that the hOOR is O-glycosylated, we first performed metabolic labeling with [3H]glucosamine (Fig. 5) that labels both N-linked and O-linked oligosaccharides. This is a technique that has been successfully used previously to demonstrate that some cell surface receptors contain O-linked glycans (42, 45). After labeling, the immunoprecipitated receptors were subjected to glycosidase treatment to remove the N-linked oligosaccharides. As can be seen in Fig. 5A (lane 1), label was detected in bands of M, 45,000 and M, 55,000, corresponding to the precursor and the mature forms of the hOOR. PNGase F treatment did not completely abolish the label from the [3H]glucosamine-labeled mature receptor because the M, 43,000 receptor species that resulted from N-
deglycosylation of the M₄ 55,000 form still contained 10–15% of the label (Fig. 5A, lane 3). This strongly suggests that the mature hδOR indeed contains O-linked oligosaccharides. Furthermore, because PNGase F was able to completely remove the label from the precursor (compare lane 3 in Fig. 5, A and B), it can be concluded that the addition of the O-linked oligosaccharides occurs after the receptor has been transported to the Golgi. Consistent with this notion, deglycosylation of the immunoprecipitated receptors after [³⁵S]methionine/cysteine pulse-chase labeling revealed that the kinetics of acquisition of Endo H-resistance and the addition of O-linked oligosaccharides (conversion of the M₄ 39,000 N-deglycosylated receptor to the M₄ 43,000 one) were indistinguishable (data not shown). The remaining ³H label in the Endo H-treated precursor (Fig. 5A, lane 2) can be explained by the fact that in contrast to PNGase F that releases intact O-linked oligosaccharides from glycoproteins, Endo H leaves one N-acetyl-galactosamine residue attached to the protein (37).

As a second approach to determine whether the hδOR contains O-linked oligosaccharides, we subjected the [³⁵S]methionine/cysteine-labeled receptor to enzymatic deglycosylation using O-glycosidase (Fig. 6). This is an enzyme that is able to remove unsubstituted O-linked galactose-N-acetyl galactosamine disaccharides from glycoproteins (46) after the terminal sialic acids have been removed by neuraminidase. To follow the subtle changes in the migration of the receptor in SDS-PAGE after the enzyme treatment, cells were treated with tunicamycin to prevent addition of N-linked glycans to the receptor. The major receptor form that was immunoprecipitated from tunicamycin-treated cells after a 5-h chase (Fig. 6, lane 2) comigrated with the N-deglycosylated mature receptor of control cells (M₄ 43,000) (Fig. 6, lane 1). Neuraminidase was able to decrease the molecular weight of the M₄ 43,000 receptor species only slightly (Fig. 6, lane 4), but a sequential treatment with neuraminidase and O-glycosidase resulted in a clear decrease in its apparent molecular weight down to M₄ 39,000 (Fig. 6, lane 6). O-Glycosidase by itself was without any effect (Fig. 6, lane 5). These results confirm that the hδOR contains O-linked oligosaccharides and further indicate that these glycans are sialylated species with a core disaccharide of galactose and N-acetylgalactosamine.

Having demonstrated that the hδOR is modified by the addition of O-linked oligosaccharides, apparently after exit from the ER, we next proceeded in characterizing further in which subcompartment of the secretory pathway this modification occurs. To this end, HEK-293S cells expressing the receptor were labeled with [³⁵S]methionine/cysteine in the presence of either monensin or BFA (Fig. 7). These drugs have an effect on protein transport through the secretory pathway, and they interfere with the processing of the N-linked as well as of the O-linked oligosaccharides. They prevent terminal processing of oligosaccharides that occurs in the trans-Golgi cisternae and the trans-Golgi network, respectively. Monensin induces neutralization of the trans-Golgi, which affects the activity of the glycosyltransferases located there (47), whereas BFA induces a rapid disassembly of the Golgi and redistribution of its resident proteins into the ER. These proteins include enzymes that normally reside in the cis, medial and trans-Golgi cisternae but none of those that reside in the trans-Golgi network (48–50). As seen in Fig. 7A (lanes 4–6), the apparent molecular weight of the receptor after the 5-h chase period was clearly smaller in monensin- and BFA-treated cells compared with control cells. This suggests that the processing of the oligosaccharides was not complete, although the M₄ 46,000 receptor species had undergone trimming of its N-linked oligosaccharides, as evidenced by its resistance to Endo H treatment (Fig. 7B, lanes 4–6). To find out, if processing of both the N-linked and the O-linked glycans was affected, the N-linked oligosaccharides were removed by treating the immunoprecipitated receptors with PNGase F (Fig. 7C, lanes 4–6). The apparent molecular weight of the N-deglycosylated receptor in BFA-treated cells was identical (M₄ 43,000) to that in control cells (compare lanes 4 and 6, Fig. 7C), indicating that the final processing of the O-linked oligosaccharides of the hδOR occurs before the protein enters the trans-Golgi network. In contrast, the final processing of the N-linked glycans of the receptor requires transport to the trans-Golgi network (compare lanes 4 and 6, Fig. 7A). The N-deglycosylated receptor of monensin-treated cells (M₄ 40,000) migrated more quickly than the N-deglycosylated receptor of BFA-treated cells (M₄ 43,000) (compare lanes 5 and 6, Fig. 7C) but slightly more slowly than the

**FIG. 5.** Metabolic labeling of the hδOR with [³H]glucosamine. HEK-293S-hδOR-FLAG cells were pulse-labeled for 60 min with [³⁵S]methionine/cysteine and chased for 5 h. The cells were treated with (lanes 2–6) or without (lane 1) tunicamycin (5 μg/ml) by adding the drug to the medium 3 h prior to the labeling. The immunoprecipitated receptors were treated with glycosidases as described under “Experimental Procedures” and subjected to SDS-PAGE and fluorography.

**FIG. 6.** Deglycosylation of the hδOR by sequential neuraminidase and O-glycosidase treatment. HEK-293S-hδOR-FLAG cells were pulse-labeled for 60 min with [³⁵S]methionine/cysteine and chased for 5 h. The cells were treated with (lanes 2–6) or without (lane 1) tunicamycin (5 μg/ml) by adding the drug to the medium 3 h prior to the labeling. The immunoprecipitated receptors were treated with glycosidases as described under “Experimental Procedures” and subjected to SDS-PAGE and fluorography.
The results obtained in the present study allow us to propose the following model for the synthesis, maturation, and plasma membrane targeting of the hδOR. The protein is initially synthesized as a M₉ 45,000 precursor that is converted in the Golgi to the mature M₉ 55,000 receptor with a half-time of about 120 min. This conversion involves processing of two high mannose-type N-linked oligosaccharides as well as addition of O-linked glycans containing N-acetylgalactosamine, galactose, and sialic acid. The fully mature receptor is then transported to the cell surface without delay in about 10 min. Once at the cell surface, the mature receptor is very stable with a half-life of about 20 h. Intriguingly, the overall process of maturation of the receptor was found to be inefficient as only 40% of the precursor was converted to the mature form and eventually reached the cell surface. This low efficiency most likely results from slow folding and ER export steps.

N-Glycosylation is a well-characterized modification of GPCRs, but O-glycosylation has gone unnoticed until very recently, when Sadeghi and Birnbaumer (51) showed that the V₂ vasopressin receptor contains O-linked glycans. In the present study, O-glycosylation of the hδOR was demonstrated by two different approaches: metabolic labeling using [³⁵S]methionine/cysteine and sequential enzymatic deglycosylation using neuraminidase and O-glycosidase. There are several reasons that could explain why the presence of O-linked oligosaccharides in the GPCRs has, until now, eluded firm biochemical identification. First, parameters that determine sites of O-glycan attachment to glycoproteins are poorly understood, and no obvious consensus sequence motif has emerged (52). This is in clear contrast to N-glycosylation for which a well-defined consensus motif has been determined (53). Another reason that may have hindered the study of O-glycans is that they have no common core structure. This is related to the fact that O-glycosylation starts post-translationally with the addition of a single monosaccharide catalyzed by several different glycosyltransferases, whereas N-glycosylation is initiated in the ER by the co-translational addition of a large oligosaccharide unit to the polypeptide by a unique oligosaccharyltransferase. The initial reaction in O-linked oligosaccharide biosynthesis is most commonly the transfer of N-acetyl-galactosamine to a serine or threonine residue on the protein acceptor. However, this is not always the case, and, for example, fucose-type O-glycosylation has also been described (54). The analysis of O-linked oligosaccharides has also been hampered by the poor availability of O-glycosylation inhibitors and of enzymes that can selectively remove these types of oligosaccharides from glycoproteins. Finally, GPCRs are likely to belong to those glycoproteins that contain only a limited number of O-linked oligosaccharides. Their primary sequences do not show extensive clusters of putative acceptor serine and threonine residues on their extracellular domains (2), a feature that is typical for many heavily O-glycosylated membrane proteins, such as the low density lipoprotein receptor (45) and the glycoporphins (55).

In contrast to the N-linked oligosaccharides, the biosynthesis and topology of which are well documented (53), the site of initiation of O-glycosylation is still somewhat controversial. Whereas the elongation steps of O-glycosylation are commonly believed to occur in the Golgi, the addition of the first monosaccharide has been placed in the ER, the ER-Golgi intermediate compartment, or the Golgi (56–59), possibly reflecting variations in the N-acetyl-galactosamine transferase localization among different cell types. Our data indicate that the O-glycosylation of the hδOR in the HEK-293S cells is initiated after the protein exits the ER, because the receptor precursor was not O-glycosylated. It was shown to contain O-linked glycans only when Golgi enzymes were allowed to redistribute into the ER by treating the cells with BFA. The data obtained using monensin-mediated interference in protein transport were consistent with the idea that the site of addition of the first O-linked monosaccharides occurs in the cis or the medial com-
portments of the Golgi. Furthermore, our results also showed that the final processing of the O-linked and the N-linked glycans of the hOOR occurs in different compartments of the cell. For the N-linked glycans, this step occurs in the trans-Golgi network, whereas for the O-linked ones it takes place in an earlier compartment, the trans-Golgi cisternae. These findings agree with those reported by Kirnje Locke et al. (43) for the coronavirus M protein and vesicular stomatitis virus G protein, showing that the terminal processing of the O-linked glycans by the addition of sialic acids occurs in the trans-Golgi, whereas the N-linked glycans are sialylated in the trans-Golgi network. The terminal processing of the oligosaccharides of the hOOR probably also involves addition of sialic acids as was evidenced by the observation that the mature receptor displays high affinity for WGA and was sensitive to neuraminidase treatment.

The kinetic data obtained from the pulse-chase labeling experiments suggest that the hOOR spends most of its intracellular residence time in the ER and is converted to a form containing O-linked glycans and Endo H-resistant N-linked glycans quite rapidly after exit from this organelle. The kinetics of oligosaccharide maturation of the receptor parallels that of the appearance of the protein at the cell surface. This implies that the time period between the removal of the mannose residues from the core oligosaccharides, the addition of the first N-acetylgalactosamine, and the subsequent transport of the protein to the plasma membrane is relatively short. Because there is no apparent lag time before the fully mature hOOR is transported to the cell surface, it is unlikely that there is a significant pool of intracellular newly synthesized mature receptors. This contrasts with findings for some other GPCRs, like the thrombin receptor (60), the N-formyl peptide receptor (61), and one of the α2-adrenergic receptor subtypes (62), for which a significant reserve of intracellular mature receptors was proposed. This may either indicate that there is an intrinsic difference between the maturation process of the hOOR and that of these receptors or else that the reserve observed in the previous studies included recycling receptors that were first targeted to the cell surface. Once at the cell surface, the mature Mf 55,000 form of the hOOR is very stable with a half-life of about 20 h. This turnover rate is somewhat slower than that reported for other GPCRs in stably transfected cells: 8–10 h and 6 h for the three α2-adrenergic receptor subtypes and the V2 vasopressin receptor, respectively (63, 64).

From the above discussion, we can conclude that export from the ER represents the rate-limiting step in the overall processing of the hOOR. In addition, this step appears to be quite inefficient because only 40% of the Mf 45,000 precursor is converted to the mature receptor form. The rest are probably retained in the ER by its quality control mechanisms and may eventually be targeted for degradation. It has been shown for several other proteins that nascent molecules that fail to fold correctly are not deployed to distal compartments of the secretory pathway and only correctly and completely folded molecules are transported out of the ER (65). One possibility that could explain the inefficient processing of the hOOR is that overexpression in the HEK-293S cells causes overloading of the capacity of the cells to properly handle the receptor protein. Several enzymes and proteins are involved in the processing and transport of nascent proteins along the secretory pathway (65, 66). It could be envisioned that in a heterologous expression system any one of these factors could become limiting, accounting for the deficiency in processing of the newly synthesized hOOR to the fully mature protein. Although this may be a reasonable explanation that cannot completely be ruled out, we consider this an unlikely possibility, because we found no correlation between the efficiency of maturation of the hOOR and the level of receptor expression in the stably transfected cells.

If saturation of a limiting factor is responsible for the inefficient processing, one would expect to observe a lower percentage of mature receptors at the higher expression level, and this was not the case. An identical efficiency of maturation was observed in cells expressing 3 and 24 pmol of receptor/mg of protein. The situation may be different, however, in transient expression systems. Indeed, Birnbaumer and co-workers observed a lower processing efficiency of the V2 vasopressin receptor when it was expressed at high levels in transient versus stable expression systems (64). In contrast, we consider it more likely that in HEK-293S cells the disposal mechanisms that are responsible for removing the misfolded receptors from the ER are overloaded, because at steady state a considerable proportion of the hOOR was in a precursor form. Similar findings have been reported for another inefficiently processed membrane protein, the cystic fibrosis transmembrane conductance regulator (67).

Given the results obtained in the present study, it is quite reasonable to hypothesize that the low efficiency of processing of the hOOR is an intrinsic property of the protein molecule itself and is related to folding difficulties. Whether this occurs in other heterologous expression systems and is a common feature among GPCRs remains to be determined. Nevertheless, it can be reasoned that the low efficiency of folding and ER export may be related to the complex structure of the GPCRs. The transmembrane domains of these proteins are believed to be arranged in a counterclockwise bundle with interhelical hydrogen bonds and a constraining disulfide bond between the first and the second extracellular loops (2–4). Complex structure of the GPCRs coupled to the need for precise intramolecular interactions may result in slow folding rate and high susceptibility to misfolding. This is exemplified by the numerous naturally occurring mutations among the GPCRs that result in intracellular trapping of the misfolded protein (12–16).

In some cases, these misfolded proteins can be rescued by incubating cells at a reduced temperature (68), indicating that folding kinetics may be important. Moreover, folding difficulties do not always follow from deviations from the wild type structure by mutations but may be typical for the wild type protein itself. For example, Fishburn et al. (69) showed that the two isoforms of the D2 dopamine receptor (D2L and D2S) are processed differently when expressed at comparable level in Chinese hamster ovary cells. The short isoform was processed fully after 3 h of chase, whereas about 20% of the long isoform were still in an immature form. Accumulation of receptor precursor forms has been shown to occur not only in heterologous expression systems but also in tissues expressing native receptors. For example, a large pool of precursor forms of the luteinizing hormone receptor and the follicle-stimulating hormone receptor was detected in testes and oocytes (70–72). Whether this also applies to the endogenous hOOR remains an open question. Unfortunately, the lack of an antibody that would allow an efficient immunopurification of the native receptor precludes realization of these experiments at the moment.

Interestingly, in some special cases normal folding and transport of GPCRs to the plasma membrane appear to involve chaperone- or escort-like proteins. This is the case for the rhodopsin in Drosophila (18, 19) and cows (20). Indeed, mutants of a specific cyclophilin-like chaperone in flies lead to accumulation of rhodopsin in the ER. It was speculated that the existence of a specific chaperone for this GPCR was necessary for optimization of the synthesis and maturation of the receptor, expressed at exceedingly high level in photoreceptor cells. Recently, distinct escort systems have also been described...
for the olfactory receptors (17), the calcitonin gene-related peptide receptor, the adrenomedullin receptor (21), and the γ-amino
butyric acid type B-1 receptor (22), indicating that the com-
plex folding of these GPCRs may require specialized proteins
even when expressed at lower level. Whether similar systems
are necessary for other GPCRs in general remains speculative.

Acknowledgments—We are grateful to Dr. Manon Valiquette for
providing us the HEK-293S cell line expressing the wild type hOR and
Dr. Robert Elde for the generous gift of the rabbit anti-hOR
antiserum. We also thank Dr. Riad Qanbar for fruitful discussions and Dr. Sultan
Ahmad for critical reading of the manuscript. The help of Huy Vu in
generating the FLAG-tagged hOR is greatly appreciated.

REFERENCES

1. Dohlman, H. G., Thorner, J., Caron, M. G., and Lefkowitz, R. J. (1991) Annu.
Rev. Biochem. 60, 653–688
2. Baldwin, J. M., Schertler, G. F. X., and Unger, V. M. (1997) J. Biol. Chem.
272, 11954–11964
3. Wess, J. (1997) FASEB J. 11, 346–354
4. Ji, T. H., Grossman, M., and Ji, I. (1998) J. Biol. Chem. 273, 17299–17302
5. Beckert, J., and Pin, J. P. (1999) EMBO J. 18, 1723–1729
6. McClintock, T. S., Landers, T. M., Gimelbrant, A. A., Fuller, L. Z., Jackson,
B. A., Jayawickreme, C. K., and Lerner, M. R. (1997) Mol. Brain Res. 48,
270–278
7. Couse, A., Filipov, A. K., Connolly, C. N., Betterly, B., Brown, D. A., and Mass,
S. J. (1998) J. Biol. Chem. 273, 26361–26367
8. Strader, C. D., Sigal, I. S., Register, R. B., Candore, M. R., Rands, E., and
Dixon, R. A. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4384–4388
9. Doi, T., Molday, R. S., and Khorana, H. G. (1990) Proc. Natl. Acad. Sci. U. S. A.
87, 4991–4995
10. Wess, J., Nanavati, S., Vogel, Z., and Maggio, R. (1993) Mol. Endocrinol.
7, 1729–1736
11. Maley, F., Trimble, R. B., Tarentine, A. L., and Plummer, T. H., Jr. (1989)
Anal. Biochem. 180, 195–204
12. Guan, X.-M., Koblika, T. S., and Koblika, B. K. (1992) J. Biol. Chem.
267, 21995–21998
13. Wiertz, E. J. H., Jones, T. B., Sun, L., Bogyo, M., Geuze, H. J., and Pliegh,
H. L. (1996) Cell 84, 769–779
14. Yu, H., Kaung, G., Kobayashi, S., Kopito, R. R. (1997) J. Biol. Chem.
272, 20803–20808
15. Bebek, Z., Mazzochi, C., King, S. A., Hong, J. S., and Sorschek, E. J. (1998)
J. Biol. Chem. 273, 29873–29878
16. Daniel, D. O., Mifley, D. F., Escobedo, J., and Williams, L. T. (1987) J. Biol.
Chem. 262, 9778–9784
17. Locker, J., Grundh, G., Horzinek, M. C., and Rottier, P. J. M. (1992) J. Biol.
Chem. 267, 14094–14101
18. Stadel, J. M., Nambi, P., Shorr, R. G. L., Sawyer, D. F., Caron, M. G., and
Lefkowitz, R. J. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 3173–3177
19. Cummings, R. D., Kornfeld, S., Schneider, W. G., Holgob, K. K., Tolleshaug,
H. M., Brown, S., and Goldstein, J. L. (1983) J. Biol. Chem. 258,
15261–15273
20. Uemoto, J., Bhashavanandan, V. P., and Davidson, E. A. (1977) J. Biol.
Chem. 252, 8609–8614
21. Mollenhauer, H. H., Moré, D. J., and Rowe, L. D. (1990) Biochem. Biophys.
Acta 1031, 225–246
22. Doms R. W., Russ, G., and Yewdell, J. W. (1989) J. Cell Biol. 109, 61–72
23. Lippincott-Schwartz, J., Yuan, L. C., Bonifacino, J. S., and Klausner, R. D.
(1989) Cell 59, 811–813
24. Chege, N. W., and Pfeffer, S. R. (1990) J. Cell Biol. 111, 893–899
25. Sadeghi, H., and Birnbaumer, M. (1999) Glycobiology 9, 731–737
26. Gooley, A. A., and Williams, K. L. (1994) Glycoconjugate J. 4, 413–417
27. Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
28. Moloney, D. J., Lin, A. I., and Haltierw, W. S. (1997) J. Biol. Chem.
272, 19046–19050
29. Pisano, A., Redmond, J. W., Williams, K. L., and Gooley, A. A. (1993) Glyco-
biology 3, 429–435
30. Pathak, R. K., Merkle, R. K., Cummings, R. D., Goldstein, J. L., Brown, M. S.,
and Anderson, R. G. W. (1988) J. Cell Biol. 116, 1831–1841
31. Piller, V., Piller, F., and Fukuda, M. (1990) J. Biol. Chem. 265, 9264–9271
32. Perez-Vilar, J., Hidalgo, J., and Velasco, A. (1991) J. Biol. Chem. 266,
23967–23976
33. Rotter, S., White, J., Wandall, H. H., Olove, J.-C., Stark, A., Bennett, E. P.,
Whitehouse, C., Berger, E. G., Clausen, H., and Nilsson, T. (1998) J. Cell
Sci. 111, 45–60
34. Heit, L., Ishii, K., Coughlin, S. R., and Koblika, B. K. (1994) J. Biol. Chem.
269, 27719–27726
35. Sengelev, H., Boulay, F., Kjeldsen, L. B., and Borregaard, N. (1994) Biochem.
J. 299, 473–479
36. von Zastrow, M., Link, R., Daunt, D., Barsh, G., and Koblika, B. K. (1993)
J. Biol. Chem. 268, 763–766
37. Wozniak, M., and Limbird, L. E. (1996) J. Biol. Chem. 271, 5017–5024
38. Rothman, J. E., and Wieland, F. T. (1996) Science 272, 227–234
39. Ward, C. L., and Kopito, R. R. (1994) J. Biol. Chem. 269, 25710–25718
40. Jaquette, J., and Segaloff, D. L. (1997) Endocrinology 138, 85–91
41. Fishburn, C. S., Elazar, Z., and Fuchs, S. (1995) J. Biol. Chem. 270,
29819–29824
42. Hipkin, R. W., Sánchez-Yagüe, J., and Aceili, M. (1992) Mol. Endocrinol.
6, 2210–2218
43. Vulfaz-Liu, T., M., Misrabi, M., Houllier, A., Jolivet, A., and Milgrom, E.
(1992) Biochemistry 31, 8377–8383
44. Vannier, B., Loosfelt, H., Meduri, G., Pichon, C., and Milgrom, E. (1996)
Biochemistry 35, 1358–1366

Downloaded from http://www.jbc.org/ on February 11, 2020
Export from the Endoplasmic Reticulum Represents the Limiting Step in the Maturation and Cell Surface Expression of the Human δ Opioid Receptor
Ulla E. Petäjä-Repo, Mireille Hogue, André Laperrière, Philippe Walker and Michel Bouvier

J. Biol. Chem. 2000, 275:13727-13736.
doi: 10.1074/jbc.275.18.13727

Access the most updated version of this article at http://www.jbc.org/content/275/18/13727

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 72 references, 37 of which can be accessed free at http://www.jbc.org/content/275/18/13727.full.html#ref-list-1