Post-translational Processing in the Golgi Plays a Critical Role in the Trafficking of the Luteinizing Hormone/Human Chorionic Gonadotropin Receptor to the Cell Surface*

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Faye A. Bradbury‡, Noritoshi Kawate§, Carol M. Foster¶, and K. M. J. Menon∫
From the Departments of Biological Chemistry and Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan 48109-0617

Point mutations in the luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptor have been shown to cause constitutive activation which results in precocious puberty in affected males. We introduced one of these mutations, Asp-556 → Gly, into the rat LH/hCG receptor and demonstrated that the mutant receptor constitutively activated adenylate cyclase in transfected 293 T cells. The cell surface expression of the mutant receptor was lower than that of the wild type receptor. Pulse-chase studies showed that the 73-kDa precursor of both the mutant and wild type receptors was synthesized at comparable efficiencies. However, post-translational processing of the mutant receptor to the mature 92-kDa form, which has N-linked complex type oligosaccharide chains, was impaired. Sensitivity of the mutant receptor to peptide-N-glycanase F and endoglycosidase H, and insensitivity to sialidase indicated that the 73-kDa species represents the high mannose form that has not yet been trafficked through the medial and trans Golgi. Additionally, although the wild type receptor was palmitoylated, the mutant receptor was not. Although the high mannose 73-kDa species is capable of binding LH/hCG, our results show that post-translational processing in the Golgi is required for the mature 92-kDa receptor to reach the cell surface.

The biological actions of luteinizing hormone/human chorionic gonadotropin (LH/hCG)1 are mediated by their interaction with specific receptors localized on the cell membranes of gonadal tissues (1). The interaction of LH/hCG with its receptor activates adenylate cyclase, resulting in an increase in cyclic AMP that stimulates steroid hormone production (2–4). The interaction of LH/hCG with its receptor stimulates steroid hormone production (2–4). The interaction of LH/hCG with its receptor activates adenylate cyclase, resulting in an increase in cyclic AMP that stimulates steroid hormone production (2–4).

EXPERIMENTAL PROCEDURES

Materials

1 The abbreviations used are: LH, luteinizing hormone; hCG, human chorionic gonadotropin; DMEM, Dulbecco’s modified Eagle’s medium; and G, protein-coupled receptors, and the deduced amino acid sequence of the LH/hCG receptor contains an extracellular domain, a seven-helix transmembrane domain, and a cytoplasmic carboxyl terminus region (5). Recently, constitutively activating mutations of the receptor have been identified that are associated with male precocious puberty (6–11). The affected males manifest pubertal development between the ages of 1 and 4 years (12, 13). One of the constitutively activating mutations involves a single base transition from A to G in the LH/hCG receptor gene. This mutation results in the substitution of glycine for aspartic acid 578 in the sixth transmembrane domain of the receptor (6).

Pulse-chase studies have shown that the LH/hCG receptor is synthesized as a precursor protein, which is processed post-translationally to a mature form of 85–92 kDa (2, 14). The post-translational events involve processing of N-linked high mannose oligosaccharides to complex N-linked oligosaccharides. Additionally, conserved cysteine residues present in the cytoplasmic tail undergo palmitoylation (2, 15). The mature receptor is then trafficked to the cell surface. Since our initial 125I-labeled hCG binding assays suggested that there were fewer mutant receptors than wild type receptors at the cell surface, we examined whether the processing and subsequent trafficking of the mutant receptor to the cell surface differs from that of the wild type receptor. We now present evidence to show that the cell surface expression of the mutant receptor is greatly reduced due to its inability to undergo processing in the Golgi.

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‡ Predoctoral fellow supported by National Institutes of Health Training Grant PR5-T32-HD-07048.
§ Present address: Dept. of Veterinary Science, Faculty of Agriculture, Yamaguchi University, Japan.
¶ Present address: Dept. of Pediatrics, University of Michigan, Ann Arbor, MI 48109-0718.
∫ To whom correspondence should be addressed: Dept. of Obstetrics and Gynecology, 6428 Medical Science I, 1300 Catherine, University of Michigan, Ann Arbor, MI 48109-0617. Tel.: 313-764-8142; Fax: 313-936-8817; E-mail: kmjmomen@umich.edu.
was removed from 60-mm dishes and the transfected cells were preincubated at 37°C in a humidified atmosphere containing 5% CO₂. Exponentially growing 293 T cells were plated 6–9 h before transfection at cell densities of 2×10⁶ cells/100-mm plate and 5–8 × 10⁵ cells/100-mm plate. The 293 T cells were transfected by a calcium phosphate coprecipitation technique with 4 or 10 μg of LH/hCG receptor cDNA constructs were selected by ampicillin screening. After confirming the mutation by DNA sequencing, the mutant cDNA was subcloned into the XhoI and SmaI sites of pCMV4. Plasmids were purified from transcripts from both RNA and DNA templates using the Qiagen 2500 columns.

Transient Expression of LH/hCG Receptor cDNA—293T cells were grown in DMEM containing 4500 mg/liter glucose, 50 μg/ml gentamicin, 2 units/ml nystatin, 10 μM Heps, and 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO₂. Exponentially growing 293 T cells were plated 6–9 h before transfection at cell densities of 2×3 × 10⁵ cells/80-mm plate and 5–8 × 10⁵ cells/100-mm plate. The 293 T cells were transfected by a calcium phosphate coprecipitation technique with 4 or 10 μg of LH/hCG receptor cDNA constructs into 60- and 100-mm plates, respectively. Mock transfections were carried out using the pCMV4 vector lacking the LH/hCG receptor cDNA. The cells were subjected to the following analyses 60 h after transfection.

125I-hCG Binding to Transfected Cells—Highly purified hCG (18) was radioiodinated with chloramine T (17). Binding of 125I-hCG to intact transfected 293 T cells was performed as described previously (2). Briefly, the cells were incubated at 4°C for 20 h (1–2 × 10⁶ cells/tube) with increasing concentrations of 125I-hCG (0.45–90 ng/ml) in the presence or absence of a 1,000-fold excess of unlabeled hCG in a final volume of 0.4 ml. All determinations were performed in duplicate, and the binding affinity and maximal binding capacity were calculated using the Ligand program and the DNA content of the cells was measured by the method of Burton (19).

125I-hCG Binding to Solubilized Transfected Cells—The binding assay using detergent solubilized extracts was carried out as described previously (20). The cells were washed free of DMEM and detached with 6 ml of ice-cold buffer A (20 mM Heps, pH 7.4, 0.15 mM NaCl) containing protease inhibitors (5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 5 mM N-ethylmaleimide, 2 μM leupeptin, and 2 μM E64). The cell suspensions (1.5 ml) were centrifuged at 12,000 × g for 15 min. The supernatant was diluted 10,000 times, and the receptor eluted by incubation at room temperature for 15 min. The solubilized extracts (0.2 ml) were incubated with 90 ng/ml 125I-hCG at 4°C for 20 h in the presence or absence of a 1,000-fold excess of unlabeled hCG in a final assay volume of 0.4 ml (see Table II). For the Scatchard analysis, the concentrations of 125I-hCG used ranged from 1 to 90 ng/ml. After the incubation, the extracts were added to Whatman GF/B filters that had been soaked at least 1 h in 0.3% polyethyleneimine in 10 mM Tris·HCl, pH 9.1 (21) and washed five times with phosphate-buffered saline containing 0.1 mM NaCl, 10 mM Na₂HPO₄, and 1 mM mg/BSA. The filters were counted in a gamma counter. Total binding was established in triplicate and nonspecific binding in duplicate. Each assay was repeated twice and the data presented are representative of the results of a typical experiment.

Cyclic AMP Measurements—Measurement of cyclic AMP followed the competitive protein binding assay of Gilman and Murad (22) as described previously by our laboratory (2). All the determinations were performed in replicates of six, and the data were analyzed using the computer program Prism (GraphPad Software, San Diego, CA).

[35S]Cysteine Labeling and Chasing of Transfected Cells—Medium was removed from 60-mm dishes and the transfected cells were preincubated at 37°C for 20 min in 6 ml of cysteine-free DMEM containing 10 mM Heps. At the end of each incubation, the samples were heated to 70°C and no EGTA. After denaturation, Endo H or sialidase was added to give a final concentration of 4 units/ml or 1.54 milliunits/ml, respectively, and the samples were incubated at 37°C for 3 h. At the end of each incubation, the samples were heated to 95°C for 2 min. Immediately prior to SDS-PAGE, the samples were boiled for 10 min.

SDS-PAGE, Fluorography, and Autoradiography—The labeled proteins were subjected to 7.5% SDS-PAGE. The 125I-cysteine- and 125I-mannose-labeled proteins were electrophoresed under nonreducing conditions; the 125I-halamic acid-labeled proteins were electrophoresed under nonreducing conditions. Fluorography was performed with Entensify™ according to the manufacturer’s protocol. The gels were then dried at 80°C for 2 h and exposed to Kodak X-Omat AR film at −70°C for the times indicated in the figure legends.

RESULTS

The conserved aspartic acid residue (556) in the sixth transmembrane domain of the rat LH/hCG receptor is analogous to aspartic acid 578 in the human LH/hCG receptor. Since the rat LH/hCG receptor is well characterized, aspartic acid 556 was mutated to glycine using site-directed mutagenesis in order to produce a constitutively active form of the receptor. The mutant receptor was cloned into a mammalian expression vector, pCMV4, and expressed in human embryonic kidney cells expressing the large T antigen (293 T). Following transient transfection, the production of cyclic AMP in response to exogenous hCG was tested. Fig. 1 shows that cells transfected with mutant LH/hCG receptor cDNA produced cyclic AMP in the absence of hCG. The mutant receptor also responds in a dose-dependent manner to increasing concentrations of hCG. At 100 ng/ml hCG, the highest concentration tested, the amount of cyclic AMP produced is similar to that seen in cells transfected with the wild type receptor. Thus, the D556G mutant receptor is constitutively activating like the D578G mutant human receptor, as has been shown previously (6). Assays of 125I-hCG binding to intact 293 T cells transiently transfected with either the wild type or mutant receptor suggest that the number of receptor sites at the cell surface is 4-fold lower than wild type (Fig. 2). However, the binding affinities (Kᵦ) of the wild type and mutant receptors are similar (Table I).

The possibility that the observed decrease in the cell surface expression of the mutant receptor was due to intracellular trapping was examined. When binding assays were performed at a saturating concentration of 125I-hCG using solubilized cells
expressing the mutant receptor, a 3-fold increase in binding activity was observed over that seen, when the binding analysis was performed using intact cells (Table II). Solubilized cells expressing the wild type receptor showed only a slight increase in binding compared to the binding activity of intact cells. The increase in binding seen in cells expressing the mutant receptor is unlikely to be due to an increase in binding affinity. Scatchard analysis of 125I-hCG binding to solubilized cells revealed that the affinities of solubilized and cell surface mutant receptors are similar, with $K_d$ values of $7.8 \times 10^{-10}$ M and $6.3 \times 10^{-10}$ M, respectively, (data not presented). These results demonstrate that the decreased cell surface expression of the mutant receptor is due to trapping of the receptor within the cell.

Since the cell surface expression of the mutant receptor was lower than the wild type receptor, we investigated the differences in the processing of the mutant receptor that lead to diminished trafficking to the cell surface. For this purpose, 293 T cells expressing the wild type or mutant receptor were labeled with $^{[35S]}$cysteine for up to 4 h. The labeled extracts were subjected to affinity chromatography on an hCG-Affi-Gel affinity matrix. An autoradiogram of the $^{[35S]}$cysteine-labeled receptor is shown in Fig. 3. A prominent band corresponding to an apparent molecular mass of 73 kDa was visible at 0 h of chase in extracts of cells expressing the wild type receptor. In extracts of cells chased with unlabeled cysteine for 2 and 4 h, respectively, a decline in the 73-kDa band occurred with a concomitant increase in the 92-kDa species. The 92-kDa species represents the fully processed, post-translational, presumably cell surface receptor. In contrast, the mutant receptor shows a very different pattern during the same chase. The bulk of the 73-kDa species was not processed into the 92-kDa form; only a faint band of molecular mass approximating the 92-kDa species was seen in extracts of cells expressing the mutant receptor. These results suggest that the mutant receptor is not processed in the same manner as the wild type receptor.

Since the mutant receptor does not appear to undergo post-translational processing to the same extent as the wild type receptor, glycosylation studies were performed to elucidate possible differences in processing. The LH/hCG receptor is known to contain at least three complex type N-linked oligosaccharide chains (24, 25). It is also known that the synthesis of complex-type N-linked oligosaccharide chains begins with the cotranslational addition of core mannose rich oligosaccharide chains to the protein. These mannose rich chains are then trimmed and new sugars are added as the protein is processed through the Golgi (26). To determine whether the mutant receptor was glycosylated, cells expressing the wild type or mutant receptor were labeled with $^{[35S]}$cysteine and chased with unlabeled cysteine for 1.5 h and chased with unlabeled cysteine for up to 4 h. The labeled extracts were subjected to affinity chromatography on an hCG-Affi-Gel affinity matrix.

### Table I

| Receptor | Intact cells | Solubilized cells | Solubilized/Intact |
|----------|--------------|-------------------|-------------------|
| $K_d$ (M) | $B_{max}$ (fmol/µg DNA) | $B_{max}$ (fmol/µg DNA) | $B_{max}$ (fmol/µg DNA) |
| Wild type | 8.5 x $10^{-10}$ | 5.97 | 1.97 |
| D556G | 5.4 x $10^{-10}$ | 1.38 | 1.38 |

### Table II

| Specific binding of 125I-hCG to intact or solubilized 293 T cells expressing wild type or D556G LH/hCG receptors
|-----------------|-----------------|-----------------|
| Receptor | Intact cells | Solubilized cells | Solubilized/Intact |
|-----------------|-----------------|-----------------|
| $B_{max}$ (fmol 125I-hCG bound/µg DNA) | $B_{max}$ (fmol/µg DNA) | $B_{max}$ (fmol/µg DNA) |
| Wild type | 5348 ± 20 | 5739 ± 228 | 1.1 |
| D556G | 1664 ± 59 | 4419 ± 142 | 2.7 |
tor contains N-linked oligosaccharide chains.

Further evidence that the mutant receptor contains N-linked mannose residues was obtained by $[^3H]$mannose labeling of cells expressing either wild type or mutant receptors. As shown in Fig. 5, the 73-kDa form of the wild type and mutant receptors incorporated $[^3H]$mannose. It is of interest that a 92-kDa form was not visible for either the wild type or mutant receptor. Taken together, the results of the PNGase F digestion and $[^3H]$mannose incorporation experiments demonstrate that the 73-kDa form of the mutant receptor contains N-linked oligosaccharides.

Since the D556G mutant receptor contains oligosaccharide chains, further studies were performed to characterize the carbohydrate chains. Cells expressing the wild type or mutant receptor were labeled with $[^35S]$cysteine for 1.5 h and chased for 2 h. After purification, the wild type and mutant receptors were treated with Endo H. Glycoproteins that have been processed beyond the endoplasmic reticulum are not expected to be sensitive Endo H (26). The 92-kDa form of the receptor was insensitive to Endo H digestion (Fig. 6). The 73-kDa form of both the mutant and wild type receptor, however, disappeared after Endo H digestion, coincident with the appearance of a 58-kDa band. Thus, susceptibility to Endo H treatment illustrates that the majority of the mutant receptor is unlikely to be processed beyond the cis Golgi.

In glycoproteins the addition of sialic acid residues to the terminal ends of oligosaccharide chains occurs in the trans Golgi stacks (27). If the 73-kDa species is a form that is not fully processed in the Golgi, it would not be expected to contain sialic acid. To examine whether the D556G mutant receptor reaches the trans Golgi stacks, hCG-Affi-Gel-purified receptor was treated with a broad action sialidase. As shown in Fig. 7, the 73-kDa form of the receptor was not sensitive to sialidase digestion. In contrast, the 92-kDa form seen in the wild type receptors was sensitive to sialidase treatment as evidenced by the disappearance of the 92-kDa form and concomitant appearance of an 82-kDa species. Therefore, the 92-kDa form of the receptor has been trafficked to the trans Golgi. These findings support the view that majority of the D556G mutant receptor is not processed in the trans Golgi stacks (27).

Another post-translational modification of the LH/hCG receptor is the palmitoylation of the conserved cysteine residues at 621 and 622 (2, 15). This post-translational modification is found on the 92-kDa form of the receptor and, therefore, is likely to occur once the receptor has left the endoplasmic reticulum. To further investigate post-translational processing of the mutant receptor, we examined whether the D556G mutant receptor could be palmitoylated like the wild type receptor. While the wild type receptor incorporated $[^3H]$palmitic acid...
into the 92-kDa form (Fig. 8), the D556G mutant did not undergo palmitoylation. These data support our hypothesis that the majority of the D556G mutant receptor does not enter the Golgi stacks, but rather remains predominantly in the endoplasmic reticulum.

**DISCUSSION**

The present study shows that, in contrast to the wild type receptor, a constitutively activating mutant of the LH/hCG receptor undergoes minimal post-translational processing. The apparent result of the decreased processing is that it leads to decreased cell surface expression. [35S]Cysteine incorporation studies demonstrate comparable levels of the 73-kDa form of the wild type and mutant receptors. However, while the wild type receptor is processed to the 92-kDa form, the mutant receptor remains largely in the 73-kDa form. The lower level of binding seen in cells expressing the mutant receptor is not due to an inability of the 73-kDa form to bind hCG as shown by the increased binding of cells expressing the mutant receptor in our solubilized receptor binding assays. Rather, the lower level of binding is due to the inability of the majority of the mutant LH/hCG receptor to reach the cell surface. These results suggest that a deficiency in processing of the mutant receptor is responsible for the lowered level of the 92-kDa form and, consequently, lower cell surface expression.

Digestion with PNGase F, which removes most N-linked oligosaccharides from denatured glycoproteins, indicates that the 73-kDa form of the receptor is glycosylated in both the wild type and mutant receptors. The glycosidase, Endo H, cleaves only mannose-rich N-linked oligosaccharide chains from proteins that have not been transported beyond the cis Golgi. The 73-kDa species, the major form of the mutant receptor, is sensitive to Endo H, suggesting that this form is not trafficked beyond the cis Golgi. In contrast, the 92-kDa form is resistant to Endo H, but sensitive to PNGase F, suggesting it is trafficked at least to the trans Golgi stacks. The observed susceptibility of the 92-kDa but not the 73-kDa species to the action of sialidase provides additional such evidence. Furthermore, in the present study, wild type receptors incorporate [3H]palmitic acid into the 92-kDa form, but little incorporation occurs in the mutant receptors. These results suggest that either the 73-kDa form does not traverse through the site where acylation occurs or that the mutant receptor is inappropriately folded and is not a substrate for palmitoylation.

In the [3H]mannose labeling experiments the 92-kDa form of the wild type and mutant receptor was not present. This absence is consistent with the fact that mannose residues are removed during post-translational processing; as a result fewer mannose residues are present in the 92-kDa form.

There are at least two possible explanations for the differences in trafficking of the wild type and mutant receptor. It has been shown that misfolded proteins are retained in the endoplasmic reticulum (28). The D556G mutation may cause improper folding of the LH/hCG receptor and thus result in much of it being trapped in the endoplasmic reticulum. Alternatively, the mutation may not affect receptor folding, but rather interrupt a sequence necessary for export from the endoplasmic reticulum. Point mutations have been found in some secreted proteins that allow the protein to fold normally, but decrease the rate at which it leaves the endoplasmic reticulum (29, 30).

In summary, we present evidence that the constitutively activating mutant LH/hCG receptor is not processed through the Golgi with the same efficiency as the wild type receptor. We suggest that the resulting low level of mutant receptors expressed on the cell surface may be sufficient to cause constitutive activation of adenylate cyclase which subsequently leads to

| CHASE TIME (H) | WT | D556G |
|---------------|----|-------|
| 2 E           | 92 kDa | 73 kDa |
| 4 6 S         | 82 kDa | 73 kDa |

**Fig. 6.** Endo H digestion of the labeled wild type and D556G LH/hCG receptors. 293 T cells transiently transfected with wild type (WT) or D556G LH/hCG receptor were pulsed with [35S]cysteine for 1.5 h and chased in medium containing unlabeled cysteine for 2 h. The labeled cells were solubilized and added to hCG-Affi-Gel columns. After elution, purified receptors were incubated in the presence or absence of Endo H (E) at 37 °C for 3 h. The samples were analyzed by SDS-PAGE under reducing conditions, and the gel was exposed to film for 2 days.

**Fig. 7.** Sialidase digestion of the labeled wild type and D556G LH/hCG receptors. 293 T cells transiently transfected with wild type (WT) or D556G LH/hCG receptor were pulsed with 2 ml of 50 μCi/ml [35S]cysteine for 1.5 h and chased in medium containing unlabeled cysteine for 4 and 6 h. The labeled cells were solubilized and added to hCG-Affi-Gel columns. After elution, purified receptors from cells that had been chased for 6 h were treated with sialidase (S) at 37 °C for 3 h. The samples were analyzed by SDS-PAGE under reducing conditions, and the gel was exposed to film for 4.5 days.
its characteristic clinical manifestations. Alternatively, incompletely processed receptor, which accumulates intracellularly, may activate $G$, resulting in constitutive activation of adenylate cyclase culminating in elevated cyclic AMP levels in the absence of LH.

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REFERENCES

1. Menon, K. M. J., and Gunaga, K. P. (1974) Fertil. Steril. 25, 732–750
2. Kawate, N., and Menon, K. M. J. (1994) J. Biol. Chem. 269, 30651–30658
3. Segaloff, D. L., and Ascoli, M. (1993) Endocrin. Rev. 14, 324–347
4. Hunzicker-Dunn, M., and Birnbaumer, L. (1985) in Luteinizing Hormone Action and Receptors (Ascoli, M., ed) pp. 57–134, CRC Press, Boca Raton, FL
5. McFarland, K. C., Sprengel, R., Phillips, H. S., Kohler, M., Rosenthal, N., Nikolov, K., Segaloff, D. L., and Seeburg, P. H. (1989) Science 245, 494–499
6. Shenker, S., Lau, L., Kosugi, S., Merendino, J. J., Jr., Takashima, M., and Cutler, G. B., Jr. (1993) Nature 365, 652–654
7. Kawate, N., Kletter, G. B., Wilson, B. E., Netzloff, M. L., and Menon, K. M. J. (1995) J. Med. Genet. 32, 553–554
8. Yano, K., Kohn, K. D., Saji, M., Okuno, A., and Cutler, G. B., Jr. (1996) Biochim. Biophys. Res. Commun. 220, 1036–1042
9. Lane, L., Chan, W-Y., Hsueh, A. J. W., Kudo, M., Hsu, S. Y., Wu, S.-H., Blomberg, L., and Cutler, G. B., Jr. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1906–1910
10. Yano, K., Sugi, M., Hidaka, A., Moriya, N., Okuno, A., Kohn, K. D., and Cutler, G. B., Jr. (1995) J. Clin. Endocrinol. Metab. 80, 1162–1168
11. Kosugi, S., Van Dop, C., Gennari, M. E., Rahl, W., Carel, J.-C., Chaussain, J.-L., Mori, T., Merendino, J. J., Jr., and Shenker, A. (1995) Hum. Mol. Genet. 4, 185–198
12. Kremer, H., Mariman, E., Otten, B. J., Moll, G. W., Jr., Stoelinga, G. B. A., Wit, J. M., Jansen, M., Drop, S. L., Faas, B., Ropers, H. H., and Brunner, H. G. (1993) Hum. Mol. Genet. 2, 1779–1783
13. Schedewie, H. K., Reiter, E. O., Betina, I. Z., Seyed, S., Wooten, V. D., Jimenez, J. F., Aiman, E. J., DeVane, G. W., Redman, J. F., and Elders, M. J. (1981) J. Clin. Endocrinol. Metab. 52, 271–278
14. Hipkin, R. W., Sanchez-Yague, J., and Ascoli, M. (1992) Mol. Endocrinol. 6, 2210–2218
15. Zhu, H., Wang, H., and Ascoli, M. (1995) Mol. Endocrinol. 9, 141–150
16. Anderson, S., Davis, D. L., Dahlback, H., Jornvall, H., and Russel, D. W. (1989) J. Biol. Chem. 264, 8222–8229
17. Azhar, S., and Menon, K. M. J. (1976) J. Biol. Chem. 251, 6548–6555
18. Munson, P. J., and Rodbard, D. (1980) Anal. Biochem. 107, 220–239
19. Burton, K. (1991) Biochem. J. 214, 315–323
20. Fernandez, L. M., and Puett, D. (1996) Biochemistry 35, 3796–3793
21. Roche, P., Bergert, E. R., and Ryan, R. J. (1985) Endocrinology 117, 790–792
22. Gilman, A. G., and Murad, F. (1974) Methods Enzymol. 38, 49–61
23. Zhang, Q. Y., and Menon, K. M. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8294–8298
24. Zhang, Z., Cai, H., Fatima, N., Buczko, E., and Dufau, M. L. (1995) J. Biol. Chem. 270, 21722–21728
25. Petaja-Repo, U. E. (1994) Biochem. J. 298, 361–366
26. Kernfeld, R., and Kernfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
27. Roth, J., Taute, D. J., Lucoq, J. M., Weinheit, J., and Paulsen, J. C. (1985) Cell 43, 287–295
28. Schatz, G., and Dobberstein, B. (1996) Science 271, 1519–1526
29. Dul, J. L., and Argon, Y. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8135–8139
30. Schauer, I., Emr, S., Gross, C., and Schekman, R. (1985) J. Cell Biol. 100, 1664–1675

FIG. 8. Incorporation of [$^3$H]palmitic acid into the wild type and D556G LH/hCG receptor. 293 T cells were transiently transfected with wild type (WT) or D556G LH/hCG receptor and labeled with [$^3$H]palmitic acid for 6 h. The labeled cells were solubilized and added to hCG-Affi-Gel columns. The eluted proteins were analyzed on SDS-PAGE under nonreducing conditions, and the gel was exposed to film for 10 days.