The Basolateral Localization Signal of the Follicle-stimulating Hormone Receptor*

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Isabelle Beau‡, Marie-Thérèse Groyer-Picard‡, André Le Bivic‡, Brigitte Vannier‡, Hugues Loosfelt‡, Edwin Milgrom‡, and Micheline Misrahi‡‡

From ‡INSERM Unité 135, Hormones Gènes et Reproduction, Institut Fédéral de Recherche 21, Hôpital Bicêtre, Assistance Publique Hôpitaux de Paris, 94275 Le Kremlin Bicêtre, France and §Laboratoire de Génétique et Physiologie du Développement, Unité Mixte de Recherche 9943, Faculté des Sciences Luminy, 13288 Marseille, France

The follicle-stimulating hormone receptor (FSHR) is physiologically localized in the basolateral compartment of the membrane of Sertoli cells. This localization is also observed when the receptor is experimentally expressed in Madin-Darby canine kidney cells. We thus used in vitro mutagenesis and transfection into these polarized cells to delineate the basolateral localization signal of the receptor.

The signal was localized in the C-terminal tail of the intracellular domain (amino acids 678–691) at a marked distance of the membrane. Mutation of individual amino acids highlighted the importance of Tyr684 and Leu689. The 14-amino acid sequence was grafted onto the p75 neurotrophin receptor and redirected this apical protein to the basolateral cell membrane compartment. Deletion of amino acids 677–695 did not modify the internalization of the FSHR, showing that the basolateral localization signal of the FSHR is not collinear with its internalization signal.

The FSH receptor (FSHR), along with the LH and TSH receptors, belongs to a subgroup of G protein-coupled receptors (reviewed in Refs. 1–5). These highly homologous proteins are unusual among G protein-coupled receptors in that they contain a very large extracellular hormone binding domain. FSH transduction pathways involve mainly Gs proteins coupling and the ensuing activation of adenylate cyclase (5).

The FSHR has a central role in reproduction through the control of gonadal development and gamete production (5). In males it is expressed in testicular Sertoli cells. These cells form an epithelial blood barrier and control the development of spermatogenesis (6). In females, the FSHR is expressed in the granulosa cells of the ovaries. It controls follicular growth and, in cooperation with the LH receptor, ovarian steroidogenesis (2).

Little is known of the intracellular trafficking of G protein-coupled receptors and, more specifically, of this subgroup of receptors. The pathways of internalization of the LH and TSH receptors have been studied at the ultrastructural level (7), but the molecular signals involved are still unknown. The LH receptor is also present in the vascular endothelium of the testes (8). This endothelial LH receptor is involved in receptor-mediated hormone transcytosis, leading to the accumulation of the hormone in close proximity to the target cells. FSHR has been detected in the vascular endothelial cells of the testes (9), but its role in hormone transcytosis has not been studied to date.

Another particularity of gonadotrophin and thyrostimulin receptors is their polarized cellular expression in several target tissues. Immunocytochemical studies with monoclonal antibodies have shown that the FSH receptor in Sertoli cells and the TSH receptor in thyroid follicular cells have a polarized basolateral expression (9, 10). By contrast, the LH receptor is expressed circumferentially in the ovarian granulosa and theca cells and in the testicular Leydig cells (11, 12). This difference in receptor distribution could result from either a difference in the structure of the receptors or from differential expression in polarized or nonpolarized cells. To distinguish between these possibilities, we have transfected the three receptors into polarized MDCK cells (13). We observed that all three receptors were directly delivered to the basolateral membrane of these cells, suggesting that they all contain a basolateral targeting signal.

The molecular signals involved in the polarized targeting of G protein-coupled receptors and more generally of hormone receptors in epithelial cells have still not been identified. We have thus undertaken to delineate this signal in the FSHR receptor. In vitro mutagenesis was followed by establishment of permanent MDCK cell lines expressing mutated receptors. We report here the localization of the basolateral targeting signal, its structure, the functional importance of the individual amino acids, and the noncollinearity of this signal with the signal for receptor endocytosis.

MATERIALS AND METHODS

Expression Vectors Encoding Deletion Mutants of FSHR—The vector encoding the wild-type FSH receptor cDNA (pSG5-FSHR) has previously been described (9). Polymerase chain reaction-mediated oligonucleotide mutagenesis was used to introduce stop codons into the pSG5-FSHR vector to produce vectors encoding FSHRΔ657–695 and FSHRΔ677–695.

In the case of the FSHRΔ657–695, a 821-base pair fragment (positions 1244–2065 of the cDNA sequence, +1 being the first base of the initiation codon) containing a stop codon at residue 657 was constructed. After digestion with PstI/NI, the purified fragment was subcloned into the pSG5-FSHR vector previously digested with the same enzyme.

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† To whom correspondence should be addressed: Hôpital de Bicêtre, INSERM U.135, 3ème niveau, 78 Rue du Général Leclerc, 94275 Le Kremlin Bicêtre, France. Tel.: 33-1-45-21-33-29; Fax: 33-1-45-21-38-22.

‡ The abbreviations used are: FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyrotrophin; FSHR, follicle-stimulating hormone receptor; MDCK, Madin-Darby canine kidney; PBS, phosphate-buffered saline; BSA, bovine serum albumin; NTR, neurotrophin receptor; NTRt, truncated neurotrophin receptor; DMEM, Dulbecco’s modified Eagle’s medium.

‡‡ C. Baratti-Elbaz, N. Ghinea, H. Loosfelt, C. Pichon, and E. Milgrom, unpublished results.
In the case of the FSHRΔ677–695, the same strategy was used. A 486-base pair fragment (positions 1793–2279 of the cDNA sequence) containing a stop codon at residue 677 was digested with Sapl and EcoRI. It was cloned into the pSG5-FSHR vector previously digested with Sapl and EcoRI.

The FSHRΔ692–695 mutant was prepared by generating a KpnI site at position 3098 of pSG5-FSHR using the same strategy as described above for the FSHRΔ677–695 mutant. This mutation yields a conservative substitution of threonine 682 for a serine. This substitution does not alter the polarity or the functional properties of the receptor. A synthetic double strand mutated oligonucleotide was then inserted between the KpnI and the EcoRI sites.

All of the constructs were verified by double-stranded DNA sequencing.

**Site-directed Mutagenesis of Receptor Basolateral Localization Signal**—Alanine scanning mutagenesis of the FSH receptor basolateral signal was performed using a series of synthetic oligonucleotides inserted between two restriction sites flanking the signal. The oligonucleotides were introduced either between the Sapl (position 3075 of the pSG5-FSHR vector) and the KpnI sites or between the KpnI and the EcoRI (position 3145) sites.

The mutants Gly681 → Ala and Ser682 → Ala were constructed using polymerase chain reaction-mediated oligonucleotide mutagenesis. A 486-base pair fragment (positions 1793–2279 of the cDNA sequence) containing the Gly681 → Ala or the Ser682 → Ala substitution was digested with Sapl and EcoRI, purified, and cloned into the Sapl-EcoRI-digested pSG5-FSHR.

**Generation of the Truncated Neurotrophin Receptor (NTRt)/FSHR**—(678–691) Hybrid Receptor—The neurotrophin receptor (NTR) cDNA, cloned in the pCB6 vector, which lacks its intracellular domain (six residues downstream the transmembrane domain), has previously been described (14). The basolateral localization signal of the FSHR was inserted into the XhoI site. The stop codon TAG, located just before the basolateral signal, was then mutated to a GAG codon encoding a glutamine using the same strategy as described above, yielding the NTR/FSHR-(678–691) hybrid receptor.

The control vector (NTRt) that was used contained a stop codon just before the first residue of the signal.

**Cell Culture and Expression of FSHR Mutants**—MDCK cells (type II) were seeded and grown on coverslips (Nunc) or filters (0.4-μm polycarbonate Transwell Costar Corp., Cambridge, MA) as described previously (13). The cells were cotransfected with expression vectors encoding either the wild-type or the mutated FSHR and with the plasmid pSV-neo, which confers resistance to the antibiotic G418. The clones expressing either the wild-type or the mutated FSHR and with the plasmid pSV-neo, which confers resistance to the antibiotic G418. The clones containing the Gly681 → Ala and Ser682 → Ala substitution were digested with Sapl and EcoRI, purified, and cloned into the Sapl–EcoRI-digested pSG5-FSHR.

**Surface Immunoprecipitation of Wild-type and Mutated FSH Receptor Complexes**—Polarized monolayers of MDCK cells were obtained after 2 days of culture on 24-mm filters. Receptors present on the cell surface were immunoprecipitated as described previously (13). Briefly, cells were pulse-labeled for 1 h with 1 μCi/ml Express 125I (NEN Life Science Products) and chased for 3 h in the same medium containing unlabeled 125I. The monoclonal antibody 64G4 was added to the apical or to the basolateral compartment during the last hour of the chase period. Extraction and purification of receptor-antibody complex were performed as described (13). All experiments were performed at least twice with triplicate samples.

**Surface Immunoprecipitation of the NTRt/FSHR-(678–691) Chimeric Receptor**—MDCK cells grown on filters were incubated for 30 min in DMEM with 10% fetal bovine serum and pulsed for 30 min in the same medium containing 1 μCi/ml [35S]cysteine (NEN Life Science Products) as described (18). After washing with DMEM, the cells were chased for 3 h in DMEM containing unlabeled amino acids.

Cell surface immunoprecipitation was performed as described (19). Briefly, the monoclonal antibody ME20.4 directed against the ectodomain of the human neurotrophin receptor was added either in the apical or in the basolateral compartment (5 μL of ascites fluid) for 1 h at 4 °C. After the incubation, the filters were washed five times for 10 min with DMEM containing 0.5% BSA. Receptor-antibody complexes were extracted and purified as described (18).

**Electrophoresis and Autoradiography**—SDS-polyacrylamide gel electrophoresis was performed as described (13). The gels were fixed and processed for fluorography. Densitometric scanning was used for quantification.

**Receptor-mediated FSH Internalization**—COS-7 cells were transfected with wild-type FSHR or the truncated FSHRΔ677–695 using Superfect (Qiagen, Chatsworth, CA). Internalization from the cell surface of wild-type FSHR or of the truncated FSHRΔ677–695 was determined by using 125I-labeled FSH (NEN Life Science Products, 130 μCi/g, 26 μCi/ml). COS-7 cells plated on six-well plates were quickly cooled to 4 °C using two washes with ice-cold PBS, 1% BSA. The cells were incubated with 125I-FSH (7000 cpm/sample in PBS, 1% BSA) for 10 min at 37 °C. The cells were then extensively washed at 4 °C to remove unbound ligand and were allowed to internalize the ligand for various time periods at 37 °C. One set of plates that were kept at 4 °C was used to measure the initial FSH binding. The internalization was stopped by rapid cooling to 4 °C. The ligand remaining on the cell surface was stripped with 50 μg/ml pepsin in PBS containing 1% BSA, pH 2, for 10 min at 37 °C. Finally, the cells were treated with 0.2% SDS, and the total radioactivity was counted. Nonspecific binding was determined in the presence of an excess of unlabeled FSH (Serono) and with nontransfected COS-7 cells. Internalized ligand was expressed as the percentage of the total radioactivity (corrected for nonspecific binding) initially bound to the cells.

Internalization was also studied in MDCK cells permanently expressing the wild-type FSHR or the truncated FSHRΔ677–695. The cells were seeded on filters and incubated 48 h later with 125I-FSH (10 μCi/ml) added to the basolateral compartment (10 min at 37 °C). The MDCK cells were then processed as described above for the COS-7 cells.

**Secondary Structure Predictions**—Secondary structure predictions were performed using computer modeling. Two methods were used: the protein sequence analysis method (20) and neural network prediction (21).

**RESULTS**

**The C-terminal Region of the Intracellular Domain of FSHR Is Involved in Basolateral Sorting**—To establish whether the intracellular domain of the FSH receptor contains basolateral sorting information, we constructed three mutants deleted for various parts of the C-terminal tail of the receptor (Fig. 2A). The truncated receptors were stably expressed in MDCK cell lines. For each mutant, several (at least five) independent clones exhibiting different levels of expression were analyzed with the anti-FSHR receptor monoclonal antibodies by confocal microscopy (Fig. 1). Monoclonal antibodies BC11 (Fig. 1a) and BB28 (Fig. 1b) that recognize the ectodomain of endogenous antigens restricted to the basolateral and the apical compartments of MDCK cell membrane, respectively (13), were used as controls.

In the first mutant (FSHRΔ657–695), the major part of the intracellular domain of the receptor was deleted. The cell lines

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The text continues with further details and results, but the complete content cannot be transcribed here due to the length and complexity of the scientific discussion.
expressing mutant or wild-type receptors were incubated with the anti-FSHR ectodomain antibody in the presence of EGTA (Fig. 1). This compound opens the tight junctions (13) and thus the anti-FSHR ectodomain antibody in the presence of EGTA expressing mutant or wild-type receptors were incubated with 

To quantify the relative proportion of receptor targeted to each membrane domain at steady state, we performed surface immunoprecipitation experiments. MDCK cells expressing wild-type or mutated receptors were pulse-labeled with 

Analysis of the kinetics of appearance of the newly synthesized protein on the basolateral and apical cell surfaces allows us to distinguish between these mechanisms (15, 19, 24–26). Such an analysis was performed by metabolic labeling of the cells followed by selective surface immunoprecipitation at various time intervals. As shown in Fig. 3, after 30 min of chase,

very likely to contain basolateral sorting information.

To narrow down the sequences of the cytoplasmic tail of the FSHR necessary for basolateral targeting, we next analyzed by confocal microscopy two additional deletion mutants: FSHRΔ657–695 and FSHRΔ692–695. While the first mutant exhibited mainly an apical localization very similar to that of the FSHRΔ657–695 mutant (Fig. 1c), the second mutant, FSHRΔ692–695, had a predominant basolateral polarized expression indistinguishable from the wild-type receptor (Fig. 1f). Thus, the sequence located between residues 678 and 691 is

The localization of the basolateral sorting signal by surface immunoprecipitation of wild-type (WT) and truncated FSHR mutants in MDCK cells. A, the sequence of the intracellular domain of the FSHR is represented. An arrow on the upper part of the graph indicates the C-terminal limit of the region highly conserved between gonadotrophin and TSH receptors. The localization of the stop codons introduced in deletion mutants (FSHRΔ657–695, FSHRΔ677–695, and FSHRΔ692–695) is indicated by arrows. B, transfected MDCK cells grown on filters were pulsed with [35S]methionine and [35S]cysteine for 1 h and chased for 3 h with a medium containing unlabeled amino acids. During the chase, an antibody specific for the extracellular domain of the FSHR was added either in the apical (Ap) or in the basolateral (Bl) compartment. The antibody-receptor complexes were purified (see “Materials and Methods”), and the radiolabeled receptor was analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. Molecular mass markers (kDa) are indicated on the left. Note that a variable proportion of the precursor high mannose receptor localized in the endoplasmic reticulum coprecipitates with the mature receptor present on the cell surface. This observation has previously been reported and ascribed to the tendency of these receptors to aggregate during the purification process (15).

FIG. 1. Localization of the basolateral sorting signal. Confocal microscopic examination of the distribution of FSHR deletion mutants in polarized MDCK cells is shown. Polarized monolayers of cells were grown to confluence on coverslips. They were treated with EGTA. The cells were processed for indirect immunofluorescence microscopy as described under “Materials and Methods.” Primary antibodies corresponded to monoclonal antibodies BC11 (a) and BB18 (b) recognizing, respectively, a basolateral and an apical endogenous antigen of MDCK cells. Monoclonal antibody FSHR 323 was also used to analyze the wild-type receptor, a strong labeling of the endoplasmic reticulum coprecipitates with the mature receptor present on the cell surface. This observation has previously been reported and ascribed to the tendency of these receptors to aggregate during the purification process (15).

FIG. 2. Localization of the basolateral sorting signal. Analysis by surface immunoprecipitation of wild-type (WT) and truncated FSHR mutants in MDCK cells. A, the sequence of the intracellular domain of the FSHR is represented. An arrow on the upper part of the graph indicates the C-terminal limit of the region highly conserved between gonadotrophin and TSH receptors. The localization of the stop codons introduced in deletion mutants (FSHRΔ657–695, FSHRΔ677–695, and FSHRΔ692–695) is indicated by arrows. B, transfected MDCK cells grown on filters were pulsed with [35S]methionine and [35S]cysteine for 1 h and chased for 3 h with a medium containing unlabeled amino acids. During the chase, an antibody specific for the extracellular domain of the FSHR was added either in the apical (Ap) or in the basolateral (Bl) compartment. The antibody-receptor complexes were purified (see “Materials and Methods”), and the radiolabeled receptor was analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. Molecular mass markers (kDa) are indicated on the left. Note that a variable proportion of the precursor high mannose receptor localized in the endoplasmic reticulum coprecipitates with the mature receptor present on the cell surface. This observation has previously been reported and ascribed to the tendency of these receptors to aggregate during the purification process (15).
FIG. 3. Time course of delivery onto the basolateral and apical membranes of FSHR receptor deleted of its basolateral localization signal. MDCK cells expressing the FSHRΔ677–695 were grown on filters, pulse-labeled for 1 h, and chased (0–3 h) at 37 °C with a medium containing unlabeled amino acids. FSHR 323 antibody was added in the apical (Ap) or in the basolateral (Bl) compartment during the last hour of the incubation. The antibody-receptor complexes were purified (see “Materials and Methods”), and the radiolabeled receptor was analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. Molecular mass markers (kDa) are indicated on the left.

TABLE I

Point mutations in the basolateral targeting signal of the FSH receptor

| Receptor     | Sequence                | Apical receptor |
|--------------|-------------------------|-----------------|
| FSHR wild type | VTSGLTYILVPLSH         | %               |
| FSHR V678A   | A-----------------------| 20              |
| FSHR T679A   | A-----------------------| 20              |
| FSHR S680A   | A-----------------------| 20              |
| FSHR G681A   | A-----------------------| 20              |
| FSHR S682A   | A-----------------------| 25              |
| FSHR T683A   | A-----------------------| 20              |
| FSHR Y684A   | A-----------------------| 20              |
| FSHR L685A   | A-----------------------| 20              |
| FSHR L686A   | A-----------------------| 20              |
| FSHR V687A   | A-----------------------| 20              |
| FSHR P688A   | A-----------------------| 25              |
| FSHR L689A   | A-----------------------| 25              |
| FSHR S680A   | A-----------------------| 20              |
| FSHR H691A   | A-----------------------| 35              |

The newly synthesized FSH receptor Δ677–695 was already detected on the apical surface of MDCK cells. A minority of receptor molecules (~25%) were also delivered to the basolateral surface of MDCK cells at this time. This was in marked contrast to what has been previously observed for the wild-type receptor, which was mainly detected on the basolateral membrane of MDCK cells in the same conditions (13). Throughout the chase phase, delivery of the mutant receptor to each surface remained constant at each time period. Approximately 60–70% of the labeled receptor molecules were found on the apical surface. There was no indication that the truncated receptor presented an increased transcytosis or decreased anchorage at the basolateral surface of the cells. This experiment thus suggested that a basolateral localization signal has been inactivated in the truncated receptor. Furthermore, the fact that the majority of receptor molecules were delivered to the apical cell surface indicates that apical sorting signal(s) may be present elsewhere in the FSHR protein and be revealed by the deletion of basolateral targeting sequences.

Individual Amino Acids Involved in the Basolateral Localization Signal—To define precisely the amino acids involved in receptor basolateral targeting, we mutated each one of the 14 residues of the 678–691 sequence into an alanine (Table I). The polarized expression of the receptor mutants was analyzed by confocal microscopy and quantified by cell surface immunoprecipitation. As shown in Figs. 4 and 5, the main effects were observed with mutations of Tyr684 and Leu689. The corresponding receptor mutants exhibited a 55 and 45% delivery to the apical surface of MDCK cells, respectively (Fig. 5). More limited effects were observed when residues in the vicinity of Tyr684 and Leu689 were mutated; about 35% of Gly681, Ile685, and His684 mutant receptors were detected at the apical surface of MDCK cells (Fig. 5 and Table I). Basolateral localization was not altered by point mutations of serine and threonine into alanine, indicating that phosphorylation of these amino acids is not involved in the vectorial sorting of the FSHR.

In conclusion, we have established that the basolateral signal of the FSH receptor comprises approximately 14 residues from positions 678–691. The activity of this signal is mainly dependent on Tyr684 and Leu689, while other residues play a less important role. Protein structure predictions (20, 21) suggest the existence of a β-turn starting at Thr680 and involving four residues (see Table I). It is followed by a β-sheet involving Tyr684–Val688. Tyrosine residues and β-turn have been shown
to have a role in several other basolateral localization signals (19, 24–29).

The FSHR Contains a Basolateral Targeting Signal That Is Autonomous and Transferable to a Heterologous Protein—We have mapped within the C-terminal end of the intracellular tail of the FSHR a sequence corresponding to residues 678–691 that was very likely to correspond to a basolateral sorting signal. Indeed, the deletion or mutation of this sequence altered the basolateral expression of the receptor. However, it remained possible that this sequence was only a part of the signal and thus necessary but not sufficient to target the receptor to the basolateral membrane. To establish unequivocally that amino acids 678–691 corresponded to an autonomous basolateral signal sequence, it was necessary to demonstrate that this polypeptide could redirect a heterologous protein to the basolateral domain. We thus grafted this sequence onto an apical sorting signal and thus necessary but not sufficient to target the receptor to the basolateral membrane.

A hybrid receptor NTRt/FSHR-(678–691) was constructed, and the polarized expression of the hybrid receptor was studied by confocal microscopy and surface immunoprecipitation in parallel with that of the NTRt. Confocal microscopy confirmed the presence of NTRt at the apical surface of MDCK cells. In contrast, the chimera NTRt/FSHR-(678–691) was detected at the basolateral surface of the cells (Fig. 6). Quantitative surface immunoprecipitation confirmed this result (Fig. 7). While 90% of the NTRt was detected at the apical surface of the cells, about 90% of the hybrid molecules were observed on the basolateral membrane.

We can thus conclude that residues 678–691 of the FSHR encode an effective basolateral targeting signal. This signal can function independently and can be transferred to another membrane protein. Furthermore, this signal is a dominant sorting signal, since apical sorting information is known to be present in the extracellular domain of the neurotrophin receptor (30).

The Basolateral Localization Signal of the FSHR Is Different from Its Endocytosis Signal and Is Not Involved in Gs Protein Coupling—Several basolateral localization signals have been shown to coincide or overlap with internalization signals (reviews in Refs. 23 and 31). We thus compared the internalization of the wild-type FSHR with that of the receptor devoid of its basolateral localization signal in COS-7 cells. As shown in Fig. 8, both receptors were internalized at the same rate.

Since there could have been differences between COS-7 and MDCK cells, we repeated this experiment in the latter cells. Again there was no difference in the internalization rate of 125I-FSH mediated by wild-type receptor or FSHRΔ677–695.

We then examined the possible involvement of this region of FSHR in Gs protein coupling. The truncated FSHRΔ677–695 was transfected into COS-7 cells and challenged with hormone. Stimulation of adenylate cyclase was similar to that observed with the wild-type receptor (not shown).

DISCUSSION

We have previously shown that the FSHR displays a polarized basolateral expression in Sertoli cells of the testes (9). We went on to demonstrate that this location can also be observed in the MDCK model system (13). We have now used in vitro mutagenesis and expression in these cells to define the basolateral sorting signal of the FSHR. Deletion mutants allowed us to map the sorting sequence to the distal part of the intracellular domain at a distance of 47 amino acids from the membrane. Point mutations have allowed us to determine that this sequence comprises approximately 14 residues and is mainly...
Basolateral Targeting Signal of FSHR

The insulin receptor has been shown to be localized in the basolateral compartment of rabbit kidney cells (33) and of transfected MDCK cells (34). Among G protein-coupled receptors, the parathyroid hormone receptor, the gp130-adrenergic (36, 37), melatonin (38), serotonergic (39, 40), calcium-sensing (41), and A1 adenosine (42) receptors also display a polarized apical or basolateral localization. However, in none of these cases has the targeting signal been identified. Recently, in vitro mutagenesis experiments have been performed on the α2α-adrenergic receptor but have failed to identify its targeting signal (43). The basolateral localization signal of the FSH receptor is thus the first signal identified for a G protein-coupled receptor and more generally for a hormone receptor.

Basolateral targeting signals have been described for a viral protein (vesicular stomatitis virus glycoprotein G) (27); for cell adhesion molecules (NCAM and CD44) (44, 45); for receptors involved in the transport of ligands (transferrin (46), polymeric immunoglobulin (15), FcIgG (47), and low density lipoprotein receptor (26), and asialoglycoprotein (27, 52)); and for a growth factor receptor (epidermal growth factor receptor (48)). All of these signals are located in the vicinity of the membrane. The basolateral targeting signal of the FSH receptor is unusually localized at a distance (47 residues) from the membrane. In many cases, basolateral localization signals are colinear with internalization signals (reviewed in Ref. 31). However, it has been shown that the two sorting processes are differentially sensitive to various point mutations (28, 49, 50, 51). The basolateral localization signal of the epidermal growth factor (48), transferrin (46), and immunoglobulin (15) receptors; the distal signal of the low density lipoprotein receptor (26); and the signal of vesicular stomatitis virus glycoprotein G (27, 52) share with the FSH receptor the property of being independent of the internalization signal.

Comparison of sequences and point mutations of basolateral localization signals has not allowed us to define a clear cut consensus sequence, although in most cases either a tyrosine or a hydrophobic amino acid doublet (Leu-Leu or Leu-Val) plays a major role in these signals (reviewed in Ref. 23). Structure predictions have indicated in several cases the existence of a β-turn (53, 54). Only one basolateral localization signal has been studied by NMR (29); in the isolated polymeric immunoglobulin receptor signal, a β-turn was observed, followed by a nascent helix. Mutations that compromised this structure were shown to decrease the sorting fidelity of the protein.

In the case of the FSH receptor, both Tyr684 and Leu689 were shown to play a major role. This is thus different from the majority of known signals (see above). Only in the case of the basolateral targeting signal of the rat lysosomal glycoprotein 120 and of vesicular stomatitis virus glycoprotein G have a Tyr and an Ile been shown to be of special importance for function (49, 52). Structure predictions using several algorithms (20, 21) have indicated that the basolateral localization signal of FSHR contains a β-turn structure. Mutation of residues located within or at close proximity to this putative structure have been shown to alter the basolateral targeting of the receptor.

LH and TSH receptors also exhibit a basolateral localization in MDCK cells. These receptors are highly homologous to the FSH receptor. Mutagenesis experiments are now performed to compare their basolateral localization signals with that of the FSHR. These studies should shed light on the cellular trafficking mechanisms of G protein-coupled receptors. Furthermore, mutations of gonadotrophin and thyrostimulin receptors have been described in a variety of diseases (reviewed in Refs. 2 and 4). It is possible that in some cases such natural mutations may alter receptor cell trafficking.

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Fig. 8. 125I-FSH internalization by the wild-type receptor and the receptor lacking its basolateral localization signal (FSHR677–695). COS-7 cells transiently expressing the wild-type (circle) or the truncated (square) FSHR677–695 receptors were incubated for 10 min with 125I-FSH (see “Materials and Methods”). After washing at 4 °C, the cells were incubated at 37 °C for various periods of time. The internalized fraction corresponds to the ligand remaining associated to the cell after an acidic wash that dissociates the remaining surface-bound hormone. Inset, a similar experiment was performed in MDCK cells permanently expressing the wild-type (circle) and the truncated receptor mutant FSHR677–695 (square).
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